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(54) Title: METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR, HEPATIC, AND BONE DISEASE

(57) Abstract: The present invention relates to methods and compositions for the diagnosis and treatment of hepatic, bone, or cardiovascular disorders. Specifically, the present invention identifies 2465 genes which are differentially expressed in hepatic, bone, or cardiovascular disorder states, relative to their expression in normal, or non-hepatic, non-bone, or non-cardiovascular disorder states, and/or in response to manipulations relevant to hepatic, bone, or cardiovascular disorders. The present invention describes methods for the diagnostic evaluation and prognosis of various hepatic, bone, or cardiovascular disorders, and for the identification of subjects exhibiting a predisposition to such conditions. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of hepatic, bone, or cardiovascular disorders.

METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR, HEPATIC, AND BONE DISEASE

Related Applications

This application claims priority to U.S. Provisional Patent Application No. 60/185,942 filed on February 29, 2000, incorporated herein in its entirety by reference.

Background of the Invention

Liver Disorders

One of the most important organs in the body, the liver is specially designed to perform many essential functions, such as the excretion of harmful substances from the body. However, its distinctive characteristics and activities render it susceptible to damage from a variety of sources, and such damage can have enormous impact on a person's health. Typical liver disorders include those related to viral infection (hepatitis), cancer, cirrhosis in response to toxins (*e.g.*, alcohol), parasites, autoimmune conditions, and genetic deficiencies in one or more enzymes critical to liver function leading to, for example, biliary atresia or hemochromatosis.

In response to damage or insult to any of its cell populations, the liver will trigger an immediate response to re-establish tissue integrity. Although different mechanisms may be used, one of two possible responses are generally observed. There is either a re-generation of tissue with complete restoration of tissue architecture and function, or there is a sustained scarring of the tissue, marked by an overproduction of matrix components. This scarring, known as fibrosis, causes deterioration of liver function and can ultimately result in liver failure.

Hepatic stellate cells are the major connective tissue-producing cells in both normal and fibrotic livers. In the normal situation, stellate cells serve as vitamin A storage sites. These cells are quiescent, show little proliferative activity, and express a limited spectrum of connective tissue proteins. In injured or fibrotic livers, however, stellate cells lose their fat-droplets and change their phenotype into myofibroblast-like cells. These myofibroblast-like cells are "activated" cells, show high proliferative activity, and produce large amounts of collagens and other extracellular matrix proteins.

A compound with an anti-fibrotic effect on stellate cells or other hepatic cells will be a promising candidate molecule for the treatment of liver disorders, such as liver fibrosis and cirrhosis. However, to date, there are no truly effective therapeutic drugs for the treatment of the fibrotic condition brought about through liver injury.

Bone Disorders

Human bone is subject to constant breakdown and re-synthesis in a complex process mediated by two cell types: osteoblasts, which produce new bone, and osteoclasts, which destroy bone. The activities of these two cell types are kept under control and in proper balance by a complex network of cytokines, growth factors and other cellular signals. It is understood that a number of known bone disorders may have their genesis in aberrant control of these cells. Likewise, a considerable amount of medical research has focused on identifying the aspects of this control network which can be exploited to re-generate bone in patients with bone diseases.

Osteoporosis is one of several known degenerative bone disorders which can cause significant risk and hardship to those affected. It is generally defined as the gradual decrease in bone strength and density that occurs with advancing age, particularly among post-menopausal women. The clinical manifestations of osteoporosis include fractures of the vertebral bodies, the neck, and intertrochanteric regions of the femur, and the distal radius. Osteoporotic individuals may fracture any bone more easily than their non-osteoporotic counterparts. As many as many as 15-20 million individuals in the United States are afflicted with osteoporosis. About 1.3 million fractures attributable to osteoporosis occur annually in people age 45 and older. Among those who live to be age 90, 32 percent of women and 17 percent of men will suffer a hip fracture, primarily due to osteoporosis.

In addition to osteoporosis, there is a plethora of other conditions which are characterized by the need to enhance bone formation. Perhaps the most obvious is in the case of bone fractures, where it would be desirable to stimulate bone growth and to hasten and complete bone repair. Agents that enhance bone formation would also be useful in certain surgical procedures (*e.g.*, facial reconstruction). Other conditions which result in a deficit or abnormal formation of bone include osteogenesis imperfecta (brittle bone disease), hypophosphatasia, Paget's disease, fibrous dysplasia,

osteopetrosis, myeloma bone disease, and the depletion of calcium in bone which is related to primary hyperparathyroidism.

There are currently no pharmaceutical approaches to managing any of these conditions that is completely satisfactory. Bone deterioration associated with osteoporosis and other bone conditions may be treated with estrogens or bisphosphonates, which have known side effects, or with further invasive surgical procedures. Bone fractures are still treated exclusively using casts, braces, anchoring devices and other strictly mechanical means. More recently, surgical approaches to these types of injury utilize bovine or human cadaver bone which is chemically treated (to remove proteins) in order to prevent rejection. However, such bone implants, while mechanically important, are biologically dead (they do not contain bone-forming cells, growth factors, or other regulatory proteins). Thus, they do not greatly modulate the repair process. All of these concerns demonstrate a great need for new or novel forms of bone therapy.

Vascular Disorders

Cardiovascular disease is a major health risk throughout the industrialized world. Atherosclerosis, the most prevalent of cardiovascular diseases, is the principal cause of heart attack, stroke, and gangrene of the extremities, and thereby the principle cause of death in the United States. Atherosclerosis is a complex disease involving many cell types and molecular factors (described in, for example, Ross, 1993, *Nature* 362: 801-809). The process, in normal circumstances a protective response to insults to the endothelium and smooth muscle cells (SMCs) of the wall of the artery, consists of the formation of fibrofatty and fibrous lesions or plaques, preceded and accompanied by inflammation. The advanced lesions of atherosclerosis may occlude the artery concerned, and result from an excessive inflammatory-fibroproliferative response to numerous different forms of insult. Injury or dysfunction of the vascular endothelium is a common feature of many conditions that predispose an individual to accelerated development of atherosclerotic cardiovascular disease. For example, shear stresses are thought to be responsible for the frequent occurrence of atherosclerotic plaques in regions of the circulatory system where turbulent blood flow occurs, such as branch points and irregular structures.

The first observable event in the formation of an atherosclerotic plaque occurs when blood-borne monocytes adhere to the vascular endothelial layer and transmigrate through to the sub-endothelial space. Adjacent endothelial cells at the same time produce oxidized low density lipoprotein (LDL). These oxidized LDLs are then taken up in large amounts by the monocytes through scavenger receptors expressed on their surfaces. In contrast to the regulated pathway by which native LDL (nLDL) is taken up by nLDL specific receptors, the scavenger pathway of uptake is not regulated by the monocytes.

These lipid-filled monocytes are called foam cells, and are the major constituent of the fatty streak. Interactions between foam cells and the endothelial and SMCs which surround them lead to a state of chronic local inflammation which can eventually lead to smooth muscle cell proliferation and migration, and the formation of a fibrous plaque.

Such plaques occlude the blood vessel concerned and, thus, restrict the flow of blood, resulting in ischemia. Ischemia is a condition characterized by a lack of oxygen supply in tissues of organs due to inadequate perfusion. Such inadequate perfusion can have a number of natural causes, including atherosclerotic or restenotic lesions, anemia, or stroke. Many medical interventions, such as the interruption of the flow of blood during bypass surgery, for example, also lead to ischemia. In addition to sometimes being caused by diseased cardiovascular tissue, ischemia may sometimes affect cardiovascular tissue, such as in ischemic heart disease. Ischemia may occur in any organ, however, that is suffering a lack of oxygen supply.

The most common cause of ischemia in the heart is atherosclerotic disease of epicardial coronary arteries. By reducing the lumen of these vessels, atherosclerosis causes an absolute decrease in myocardial perfusion in the basal state or limits appropriate increases in perfusion when the demand for flow is augmented. Coronary blood flow can also be limited by arterial thrombi, spasm, and, rarely, coronary emboli, as well as by ostial narrowing due to luetic aortitis. Congenital abnormalities, such as anomalous origin of the left anterior descending coronary artery from the pulmonary artery, may cause myocardial ischemia and infarction in infancy, but this cause is very rare in adults.

Myocardial ischemia can also occur if myocardial oxygen demands are abnormally increased, as in severe ventricular hypertrophy due to hypertension or aortic stenosis. The latter can be present with angina that is indistinguishable from that caused by coronary atherosclerosis. A reduction in the oxygen-carrying capacity of the blood, as in extremely severe anemia or in the presence of carboxy-hemoglobin, is a rare cause of myocardial ischemia. Not infrequently, two or more causes of ischemia will coexist, such as an increase in oxygen demand due to left ventricular hypertrophy and a reduction in oxygen supply secondary to coronary atherosclerosis.

The principal surgical approaches to the treatment of ischemic atherosclerosis are bypass grafting, endarterectomy, and percutaneous translumenal angioplasty (PCTA). The failure rate after these approaches due to restenosis, in which the occlusions recur and often become even worse, is extraordinarily high (30-50%). It appears that much of the restenosis is due to further inflammation, smooth muscle accumulation, and thrombosis. Additional therapeutic approaches to cardiovascular disease have included treatments that encouraged angiogenesis in such conditions as ischemic heart and limb disease.

Angiogenesis is a fundamental process by which new blood vessels are formed, as reviewed, for example, by Folkman and Shing, J. Biol. Chem. 267 (16), 10931-10934 (1992). Capillary blood vessels consist of endothelial cells and pericytes. These two cell types carry all of the genetic information to form tubes, branches and whole capillary networks. Specific angiogenic molecules and growth factors can initiate this process, while specific inhibitory molecules can stop it. These molecules with opposing function appear to be continuously acting in concert to maintain a stable microvasculature in which endothelial cell turnover is thousands of days. However, the same endothelial cells can undergo rapid proliferation, i.e. less than five days, during burst of angiogenesis, for example, during wound healing.

Key components of the angiogenic process are the degradation of the basement membrane, the migration and proliferation of capillary endothelial cell (EC) and the formation of three dimensional capillary tubes. The normal vascular turnover is rather low: the doubling time for capillary endothelium is from 50-20,000 days, but it is 2-13 days for tumor capillary endothelium. The current understanding of the sequence of events leading to angiogenesis is that a cytokine capable of stimulating endothelial cell

proliferation, such as fibroblast growth factor (FGF), causes release of collagenase or plasminogen activator which, in turn, degrade the basement membrane of the parent venule to facilitate the migration of the endothelial cells. These capillary cells, having sprouted from the parent vessel, proliferate in response to growth factors and angiogenic agents in the surrounding environment to form lumen and eventually new blood vessels.

The development of a vascular blood supply is essential in reproduction, development and wound repair (Folkman, *et al.*, Science 43, 1490-1493 (1989)). Under these conditions, angiogenesis is highly regulated, so that it is turned on only as necessary, usually for brief periods of days, then completely inhibited. However, a number of serious diseases are also dominated by persistent unregulated angiogenesis and/or abnormal neovascularization including solid tumor growth and metastasis, psoriasis, endometriosis, Grave's disease, ischemic disease (*e.g.*, atherosclerosis), and chronic inflammatory diseases (e.g., rheumatoid arthritis), and some types of eye disorders, (reviewed by Auerbach, *et al.*, J. Microvasc. Res. 29, 401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, pp. 175-203 (Academic Press, New York 1985); Patz, Am. J. Opthalmol. 94, 715-743 (1982); and Folkman, *et al.*, Science 221, 719-725 (1983)). For example, there are a number of eye diseases, many of which lead to blindness, in which ocular neovascularization occurs in response to the diseased state. These ocular disorders include diabetic retinopathy, macular degeneration, neovascular glaucoma, inflammatory diseases and ocular tumors (*e.g.*, retinoblastoma). There are a number of other eye diseases which are also associated with neovascularization, including retrolental fibroplasia, uveitis, eye diseases associated with choroidal neovascularization and eye diseases which are associated with iris neovascularization.

Vascular tone refers to the degree of constriction experienced by a blood vessel relative to its maximal dilated state. All vessels under basal conditions exhibit some degree of smooth muscle contraction that determines the diameter, and hence tone, of the vessel. Basal vascular tone differs among organs wherein organs with a large vasodilatory capacity have high vascular tone (e.g., myocardium, skeletal muscle, skin), and organs with low vasodilatory capacity have low vascular tone (e.g., cerebral and renal circulatory systems).

Vascular tone is determined by many different competing vasoconstrictor and vasodilator influences acting upon the blood vessel. These influences can be separated into extrinsic factors that originate from outside of the organ or tissue where the blood vessel is located, and intrinsic factors that originate from the vessel itself or the surrounding tissue. Extrinsic factors primarily serve the function of regulating arterial blood pressure, while intrinsic mechanisms are concerned with local blood flow regulation within an organ. Vascular tone at any given instant is determined by the balance of competing vasoconstrictor and vasodilator influences.

Summary of the Invention

The present invention provides methods and compositions for the diagnosis and treatment of hepatic disease and bone associated disease, including but not limited to, liver fibrosis, hepatitis, liver tumors, cirrhosis of the liver, hemochromatosis, liver parasite induced disorders, alpha-1 antitrypsin deficiency, autoimmune hepatitis, biliary atresia osteogenesis imperfecta (brittle bone disease), osteoporosis, Paget's disease (enlarged bones), fibrous dysplasia (uneven bone growth), hypophosphatasia, osteopetrosis, primary gyperthyroidism, or myeloma bone disease. The present invention is based, at least in part, on the discovery that the 2465 gene is up-regulated in stellate cells (the main effectors of liver fibrosis) as compared to its expression in hepatic cells, and, thus, may be associated with a hepatic disorder. The present invention is further based, at least in part, on the discovery that the 2465 gene is up-regulated during osteoblast differentiation, and, thus, may be associated with a bone disorder.

The present invention is also based, at least in part, on the discovery that the 2465 gene is expressed in isolated human blood vessels (*e.g.*, in isolated endothelial vasculature cells and smooth muscle vasculature cells), and is upregulated in response to laminar shear stress, under proliferating conditions, and during treatment with IL-1β. Accordingly, the present invention also provides methods and compositions for the diagnosis and treatment of cardiovascular disease, including but not limited to, atherosclerosis, ischemia/reperfusion injury, hypertension, restenosis, arterial inflammation, and endothelial cell disorders, such as disorders associated with aberrant endothelial cell growth, angiogenesis and/or vascularization.

In one aspect, the invention provides a method for identifying the presence of a nucleic acid molecule associated with a hepatic, bone, cardiovascular, or endothelial cell disorder in a sample by contacting a sample comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1, and detecting the presence of a nucleic acid molecule associated with a hepatic, bone, cardiovascular, or endothelial cell disorder when the sample contains a nucleic acid molecule that hybridizes to the nucleic acid probe. In one embodiment, the hybridization probe is detectably labeled. In another embodiment the sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis and southern blotting prior to contacting with the hybridization probe. In a further embodiment, the sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis and northern blotting prior to contacting with the hybridization probe. In yet another embodiment, the detecting is by *in situ* hybridization. In other embodiments, the method is used to detect mRNA or genomic DNA in the sample.

The invention also provides a method for identifying a nucleic acid associated with a hepatic, bone, cardiovascular, or endothelial cell disorder in a sample, by contacting a sample comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1, incubating the sample under conditions that allow for nucleic acid amplification, and detecting the presence of a nucleic acid molecule associated with a hepatic, bone, cardiovascular, or endothelial cell disorder when the sample contains a nucleic acid molecule that is amplified. In one embodiment, the sample comprising nucleic acid molecules is subjected to agarose gel electrophoresis after the incubation step.

In addition, the invention provides a method for identifying a polypeptide associated with a hepatic, bone, cardiovascular, or endothelial cell disorder in a sample by contacting a sample comprising polypeptide molecules with a binding substance specific for a 2465 polypeptide, and detecting the presence of a polypeptide associated with a hepatic, bone, cardiovascular, or endothelial cell disorder when the sample contains a polypeptide molecule that binds to the binding substance. In one embodiment

the binding substance is an antibody. In another embodiment, the binding substance is a 2465 ligand. In a further embodiment, the binding substance is detectably labeled.

In another aspect, the invention provides a method of identifying a subject at risk for a hepatic, bone, cardiovascular, or endothelial cell disorder by contacting a sample obtained from the subject comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1, and detecting the presence of a nucleic acid molecule which identifies a subject a risk for a hepatic, bone, cardiovascular, or endothelial cell disorder when the sample contains a nucleic acid molecule that hybridizes to the nucleic acid probe.

In a further aspect, the invention provides a method for identifying a subject at risk for a hepatic, bone, cardiovascular, or endothelial cell disorder by contacting a sample obtained from a subject comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1, incubating the sample under conditions that allow for nucleic acid amplification, and detecting a nucleic acid molecule which identifies a subject at risk for a hepatic, bone, cardiovascular, or endothelial cell disorder when the sample contains a nucleic acid molecule that is amplified.

In yet another aspect, the invention provides a method of identifying a subject at risk for a hepatic, bone, cardiovascular, or endothelial cell disorder by contacting a sample obtained from the subject comprising polypeptide molecules with a binding substance specific for a 2465 polypeptide, and identifying a subject at risk for a hepatic, bone, cardiovascular, or endothelial cell disorder by detecting the presence of a polypeptide molecule in the sample that binds to the binding substance.

In another aspect, the invention provides a method for identifying a compound capable of treating a hepatic, bone, cardiovascular, or endothelial cell disorder characterized by aberrant 2465 nucleic acid expression or 2465 protein activity by assaying the ability of the compound to modulate the expression of a 2465 nucleic acid or the activity of a 2465 protein. In one embodiment, the disorder is liver fibrosis. In another embodiment, the disorder is osteoporosis. In another embodiment, the disorder is cardiovascular. In a further embodiment, the ability of the compound to modulate the

activity of the 2465 protein is determined by detecting the induction of an intracellular second messenger.

In addition, the invention provides a method for treating a subject having a hepatic, bone, cardiovascular, or endothelial cell disorder characterized by aberrant 2465 protein activity or aberrant 2465 nucleic acid expression by administering to the subject a 2465 modulator. In one embodiment, the 2465 modulator is administered in a pharmaceutically acceptable formulation. In another embodiment the 2465 modulator is administered using a gene therapy vector. In a further embodiment, the 2465 modulator is a small molecule.

In one embodiment, a modulator is capable of modulating 2465 polypeptide activity. In another embodiment, the 2465 modulator is an anti-2465 antibody. In a further embodiment, the 2465 modulator is a 2465 polypeptide comprising the amino acid sequence of SEQ ID NO:2, or a fragment thereof. In yet another embodiment, the 2465 modulator is a 2465 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4. In a further embodiment, the 2465 modulator is an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

In one embodiment, the 2465 modulator is capable of modulating 2465 nucleic acid expression. In another embodiment, the 2465 modulator is an antisense 2465 nucleic acid molecule. In yet another embodiment, the 2465 modulator is a ribozyme. In a further embodiment, the 2465 modulator comprises the nucleotide sequence of SEQ ID NO:1, or a fragment thereof. In another embodiment, the 2465 modulator comprises a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4. In yet another embodiment, the 2465 modulator comprises a nucleic

acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

In another aspect, the invention provides a method for identifying a compound capable of modulating a hepatocyte, osteocyte, or endothelial cell activity by contacting a hepatocyte, osteocyte, or endothelial cell with a test compound and assaying the ability of the test compound to modulate the expression of a 2465 nucleic acid or the activity of a 2465 protein. In certain embodiments, a compound that modulates the expression of a 2465 nucleic acid or the activity of a 2465 protein modulates hepatocyte, osteocyte, or endothelial cell proliferation, migration, or the expression of cell surface adhesion molecules.

Furthermore, the invention provides a method for modulating a hepatocyte, osteocyte, or endothelial cell activity comprising contacting a hepatocyte, osteocyte, or endothelial cell with a 2465 modulator.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 depicts the cDNA sequence and predicted amino acid sequence of human 2465 (GenBank Accession D38449). The nucleotide sequence corresponds to nucleic acids 1 to 2816 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 516 of SEQ ID NO: 2.

Figure 2 depicts a structural, hydrophobicity, and antigenicity analysis of the human 2465 protein.

Figure 3 depicts an alignment of the human 2465 polypeptide with human Accession Number D38449, and rat Accession Number D38450 using the CLUSTALW (1.74) multiple sequence alignment program.

Figure 4 is a graph depicting transcriptional profiling data of human 2465 cDNA expression in various tissues.

Figure 5 is a graph depicting the results of RT-PCR analysis of human 2465 expression in cells related to liver fibrosis.

Figure 6 is a graph depicting transcriptional profiling data of human 2465 cDNA expression in various tissues.

Figure 7 is a graph depicting the results of RT-PCR analysis of rat 2465 ortholog expression in a mouse model of liver fibrosis generated by bile duct ligation.

Figure 8 is a graph depicting the results of RT-PCR analysis of rat 2465 ortholog expression in a mouse model of liver fibrosis generated by injection of porcine serum.

Figure 9 is a graph depicting the results of RT-PCR analysis of rat 2465 ortholog expression in a mouse model of liver fibrosis generated by exposure to carbon tetrachloride.

Figure 10 is a graph depicting the results of RT-PCR analysis of human 2465 expression in an adipocyte, an osteoblast, and a progenitor lineage.

Figure 11 is a graph depicting relative expression levels of human 2465 in osteogenic versus adipogenic tissues.

Figure 12 is a graph depicting relative expression levels of human 2465 in several cellular models of osteoporosis.

Figure 13 is a graph depicting transcriptional profiling data of human 2465 cDNA expression in various tissues.

Figure 14 is a graph depicting transcriptional profiling data of human 2465 cDNA expression in isolated human vessels.

Figure 15 is a graph depicting transcriptional profiling data of human 2465 cDNA expression during the use of stimuli relevant to angiogenesis, atherosclerosis, and vascular tone.

Detailed Description of the Invention

The present invention provides methods and compositions for the diagnosis and treatment of cardiovascular, hepatic disease, and bone associated disease, including but not limited to, atherosclerosis, ischemia/reperfusion injury, hypertension, restenosis, arterial inflammation, liver fibrosis, hepatitis, liver tumors, cirrhosis of the liver, hemochromatosis, liver parasite induced disorders, alpha-1 antitrypsin deficiency, autoimmune hepatitis, biliary atresia osteogenesis imperfecta (brittle bone disease), osteoporosis, Paget's disease (enlarged bones), fibrous dysplasia (uneven bone growth), hypophosphatasia, osteopetrosis, primary gyperthyroidism, or myeloma bone disease.

The present invention is based, at least in part, on the discovery that G protein-coupled receptor genes, referred to herein as "G protein-coupled receptor 2465" or "2465" nucleic acid and protein molecules, are up-regulated in stellate cells (the main effectors of liver fibrosis) as compared to their expression in hepatic cells, and, thus, may be associated with a hepatic disorder. The present invention is further based, at least in part, on the discovery that the 2465 molecules are up-regulated during osteoblast differentiation, and, thus, may be associated with a bone disorder.

The present invention is also based, at least in part, on the discovery that the 2465 gene is expressed in isolated human blood vessels (*e.g.*, in isolated endothelial vasculature cells and smooth muscle vasculature cells), and is upregulated in response to laminar shear stress, under proliferating conditions, and during treatment with IL-1β.

As used herein, “differential expression” includes both quantitative as well as qualitative differences in the temporal and/or tissue expression pattern of a gene. Thus, a differentially expressed gene may have its expression activated or inactivated in normal versus hepatic, bone, or cardiovascular conditions (for example, in an experimental liver fibrosis disease system or a laminar shear stress system). The degree to which expression differs in normal versus hepatic, bone, or cardiovascular disorder or control versus experimental states need only be large enough to be visualized via standard characterization techniques, *e.g.*, quantitative PCR, Northern analysis, or subtractive hybridization. The expression pattern of a differentially expressed gene may be used as part of a prognostic or diagnostic hepatic, bone, or cardiovascular disorder evaluation, or may be used in methods for identifying compounds useful for the treatment of hepatic, bone, or cardiovascular disorder. In addition, a differentially expressed gene involved in hepatic, bone, or cardiovascular disorders may represent a target gene such that modulation of the level of target gene expression or of target gene product activity may act to ameliorate a hepatic, bone, or cardiovascular disorder condition. Compounds that modulate target gene expression or activity of the target gene product can be used in the treatment of hepatic, bone, or cardiovascular disorders. Although the 2465 genes described herein may be differentially expressed with respect to hepatic, bone, or cardiovascular disorders, and/or their products may interact with gene products important to hepatic, bone, or cardiovascular disorders, the genes may

also be involved in mechanisms important to additional hepatic, bone, or cardiovascular processes.

The 2465 molecules of the present invention may be involved in signal transduction and, thus, may function to modulate cell proliferation, differentiation, and motility. Thus, the 2465 molecules of the present invention may play a role in cellular growth signaling mechanisms. As used herein, the term "cellular growth signaling mechanisms" includes signal transmission from cell receptors, *e.g.*, G protein coupled receptors, which regulates 1) cell transversal through the cell cycle, 2) cell differentiation, 3) cell survival, 4) cell migration and patterning, and/or 5) cell proliferation (e.g., endothelial cell proliferation).

Accordingly, the 2465 molecules of the present invention may be involved in cellular signal transduction pathways that modulate hepatic, bone, or cardiovascular cell activity. As used herein, a "hepatic cell activity", "hepatocyte activity", or "hepatic cell function" includes cell proliferation, differentiation, migration, and expression of cell surface adhesion molecules, as well as cellular process that contribute to the physiological role of hepatic cells (*e.g.*, the regulation of bile secretion). As used herein, a "bone cell activity", "osteocyte activity", or "bone cell function" includes cell proliferation, differentiation, migration, and expression of cell surface adhesion molecules, as well as cellular process that contribute to the physiological role of bone cells (*e.g.*, the regulation of calcium secretion). As used herein, a "cardiovascular cell activity", "cardiovascular activity", or "cardiovascular function" includes cell proliferation, differentiation, migration, and expression of cell surface adhesion molecules, as well as cellular process that contribute to the physiological role of cardiovascular cells such as endothelial cells (*e.g.*, the regulation of angiogenesis and/or vascular tone).

The 2465 molecules of the present invention may act as novel diagnostic targets and therapeutic agents for hepatic diseases or disorders. As used herein, a "hepatic disorder" includes a disease or disorder which affects the liver. The term hepatic disorder includes a disorder caused by the over- or under-production of hepatic enzymes, *e.g.*, alanine aminotransferase, aspartate aminotransferase, or γ-glutammyl transferase, in the liver. For example, a hepatic disorder includes hepatic fibrosis, hepatic cirrhosis, a hepatic disorder caused by a drug, a hepatic disorder caused by

prolonged ethanol intake, a hepatic injury caused by carbon tetrachloride exposure, hepatitis, liver tumors, cirrhosis of the liver, hemochromatosis, liver parasite induced disorders, alpha-1 antitrypsin deficiency, or autoimmune hepatitis. Hepatic disorders are disclosed at, for example, the American Liver Foundation website (on the world wide web at: gi.ucsf.edu/alf.html).

The 2465 molecules of the present invention may also act as novel diagnostic targets and therapeutic agents for bone associated diseases or disorders. As used herein, a "bone associated disease or disorder" includes a disease or disorder which affects bones. The term bone associated disorder includes a disorder affecting the normal function of the bones. For example, a bone associated disorder includes biliary atresia osteogenesis imperfecta (brittle bone disease), osteoporosis, Paget's disease (enlarged bones), fibrous dysplasia (uneven bone growth), hypophosphatasia, osteopetrosis, primary gyperthyroidism, or myeloma bone disease. Bone associated disorders are described in, for example, Lamber *et al.* (2000) *Pharmacotherapy* 20:34-51; Eisman *et al.* (1999) *Endocrine Reviews* 20:788-804; Byers *et al.* (1992) *Annual Rev. Med.*, 43:269-282; and at www.osteo.org.

A hepatic, bone, or cardiovascular disorder also includes a hepatic cell or bone cell disorder. As used herein a "hepatic cell disorder" includes a disorder characterized by aberrant or unwanted hepatic cell activity, *e.g.*, proliferation, migration, angiogenesis, or aberrant expression of cell surface adhesion molecules. As used herein a "bone cell disorder" includes a disorder characterized by aberrant or unwanted bone cell activity, *e.g.*, proliferation, migration, angiogenesis, or aberrant expression of cell surface adhesion molecules.

The 2465 molecules of the present invention may also act as novel diagnostic targets and therapeutic agents for cardiovascular diseases or disorders. As used herein, "cardiovascular disease" or a "cardiovascular disorder" includes a disease or disorder which affects the cardiovascular system, e.g., the heart or the blood vessels. A cardiovascular disorder includes disorders such as arteriosclerosis, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node

dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, dilated cardiomyopathy, idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, ischemic disease, arrhythmia, and cardiovascular developmental disorders (*e.g.*, arteriovenous malformations, arteriovenous fistulae, Raynaud's syndrome, neurogenic thoracic outlet syndrome, causalgia/reflex sympathetic dystrophy, hemangioma, aneurysm, cavernous angioma, aortic valve stenosis, atrial septal defects, atrioventricular canal, coarctation of the aorta, ebsteins anomaly, hypoplastic left heart syndrome, interruption of the aortic arch, mitral valve prolapse, ductus arteriosus, patent foramen ovale, partial anomalous pulmonary venous return, pulmonary atresia with ventricular septal defect, pulmonary atresia without ventricular septal defect, persistance of the fetal circulation, pulmonary valve stenosis, single ventricle, total anomalous pulmonary venous return, transposition of the great vessels, tricuspid atresia, truncus arteriosus, ventricular septal defects).

A cardiovasular disease or disorder also includes an endothelial cell and/or smooth muscle cell disorder. As used herein, an “endothelial cell disorder” and/or a “smooth muscle cell disorder” includes a disorder characterized by aberrant, unregulated, or unwanted endothelial cell activity, *e.g.*, vascular tone, vasodilation, vasoconstriction, proliferation, migration, angiogenesis, or vascularization; or aberrant expression of cell surface adhesion molecules or genes associated with angiogenesis, *e.g.*, TIE-2, FLT and FLK. Endothelial cell disorders include tumorigenesis, tumor metastasis, psoriasis, diabetic retinopathy, endometriosis, Grave’s disease, ischemic disease (*e.g.*, atherosclerosis), chronic inflammatory diseases (*e.g.*, rheumatoid arthritis), arterial hypertension, pulmonary hypertension, primary pulmonary hypertension (PPH), Raynaud’s phenomenon (RP), migraine headache, chronic heart failure, erythromelalgia, familial dysautonomia, hemolytic uremic syndrome, preeclampsia, reperfusion injury, postangioplasty enothelial regeneration, degeneration of venous bypass grafts, angina, pure spastic angina, diabetes, reflex sympathetic dystrophy syndrome, and vasculitis.

The present invention provides methods for identifying the presence of a 2465 nucleic acid or polypeptide molecule associated with a hepatic, bone, or cardiovascular disorder. In addition, the invention provides methods for identifying a subject at risk for a hepatic, bone, or cardiovascular disorder by detecting the presence of a 2465 nucleic acid or polypeptide molecule.

The invention also provides a method for identifying a compound capable of treating a hepatic, bone, or cardiovascular disorder characterized by aberrant 2465 nucleic acid expression or 2465 protein activity by assaying the ability of the compound to modulate the expression of a 2465 nucleic acid or the activity of a 2465 protein. Furthermore, the invention provides a method for treating a subject having a hepatic, bone, or cardiovascular disorder characterized by aberrant 2465 protein activity or aberrant 2465 nucleic acid expression by administering to the subject a 2465 modulator which is capable of modulating 2465 protein activity or 2465 nucleic acid expression.

Moreover, the invention provides a method for identifying a compound capable of modulating an endothelial cell activity by modulating the expression of a 2465 nucleic acid or the activity of a 2465 protein. The invention provides a method for modulating an endothelial cell activity comprising contacting an endothelial cell with a 2465 modulator.

Various aspects of the invention are described in further detail in the following subsections.

1. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules (organic or inorganic) or other drugs) which bind to 2465 proteins, have a stimulatory or inhibitory effect on, for example, 2465 expression or 2465 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 2465 substrate.

These assays are designed to identify compounds that bind to a 2465 protein, bind to other cellular or extracellular proteins that interact with a 2465 protein, and interfere with the interaction of the 2465 protein with other cellular or extracellular proteins. For example, in the case of the 2465 protein, which is a transmembrane receptor-type protein, such techniques can identify ligands for such a receptor. A 2465 protein ligand can, for example, act as the basis for amelioration of hepatic, bone, or cardiovascular disorders, such as, for example, atherosclerosis, hypertension, liver fibrosis or osteoporosis. Such compounds may include, but are not limited to peptides,

antibodies, or small organic or inorganic compounds. Such compounds may also include other cellular proteins.

Compounds identified via assays such as those described herein may be useful, for example, for ameliorating hepatic, bone, or cardiovascular disorders. In instances whereby a hepatic, bone, or cardiovascular disorder condition results from an overall lower level of 2465 gene expression and/or 2465 protein in a cell or tissue, compounds that interact with the 2465 protein may include compounds which accentuate or amplify the activity of the bound 2465 protein. Such compounds would bring about an effective increase in the level of 2465 protein activity, thus ameliorating symptoms.

In other instances mutations within the 2465 gene may cause aberrant types or excessive amounts of 2465 proteins to be made which have a deleterious effect that leads to hepatic, bone, or cardiovascular disorders. Similarly, physiological conditions may cause an excessive increase in 2465 gene expression leading to hepatic, bone, or cardiovascular disorders. In such cases, compounds that bind to a 2465 protein may be identified that inhibit the activity of the 2465 protein. Assays for testing the effectiveness of compounds identified by techniques such as those described in this section are discussed herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 2465 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 2465 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 2465 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate 2465 activity is determined. Determining the ability of the test compound to modulate 2465 activity can be accomplished by monitoring, for example, intracellular calcium, IP_3, cAMP, or diacylglycerol concentration, the phosphorylation profile of intracellular proteins, cell proliferation and/or migration, the expression of cell surface adhesion molecules, or the activity of a 2465-regulated transcription factor or gene expression of, for example, cell surface adhesion molecules or genes associated with angiogenesis. The cell can be of mammalian origin, *e.g.*, a hepatic, bone, or endothelial cell. In one embodiment, compounds that interact with a 2465 receptor domain can be screened for their ability to function as ligands, *i.e.*, to bind to the 2465 receptor and modulate a signal transduction pathway. Identification of 2465 ligands, and measuring the activity of the ligand-receptor complex, leads to the identification of modulators (*e.g.*, antagonists) of this interaction. Such modulators may be useful in the treatment of hepatic, bone, or cardiovascular disorders.

The ability of the test compound to modulate 2465 binding to a substrate or to bind to 2465 can also be determined. Determining the ability of the test compound to modulate 2465 binding to a substrate can be accomplished, for example, by coupling the

2465 substrate with a radioisotope or enzymatic label such that binding of the 2465 substrate to 2465 can be determined by detecting the labeled 2465 substrate in a complex. 2465 could also be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 2465 binding to a 2465 substrate in a complex. Determining the ability of the test compound to bind 2465 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to 2465 can be determined by detecting the labeled 2465 compound in a complex. For example, compounds (*e.g.*, 2465 ligands or substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Compounds can further be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a 2465 ligand or substrate) to interact with 2465 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 2465 without the labeling of either the compound or the 2465 (McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 2465.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 2465 target molecule (*e.g.*, a 2465 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 2465 target molecule. Determining the ability of the test compound to modulate the activity of a 2465 target molecule can be accomplished, for example, by determining the ability of the 2465 protein to bind to or interact with the 2465 target molecule.

Determining the ability of the 2465 protein or a biologically active fragment thereof, to bind to or interact with a 2465 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred

embodiment, determining the ability of the 2465 protein to bind to or interact with a 2465 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+}, diacylglycerol, IP_3, cAMP), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response (*e.g.*, cell proliferation or migration).

In yet another embodiment, an assay of the present invention is a cell-free assay in which a 2465 protein or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the 2465 protein or biologically active portion thereof is determined. Preferred biologically active portions of the 2465 proteins to be used in assays of the present invention include fragments which participate in interactions with non-2465 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the 2465 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 2465 protein or biologically active portion thereof with a known compound which binds 2465 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 2465 protein, wherein determining the ability of the test compound to interact with a 2465 protein comprises determining the ability of the test compound to preferentially bind to 2465 or biologically active portion thereof as compared to the known compound. Compounds that modulate the interaction of 2465 with a known target protein may be useful in regulating the activity of a 2465 protein, especially a mutant 2465 protein.

In another embodiment, the assay is a cell-free assay in which a 2465 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 2465 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 2465 protein can be accomplished, for example, by determining the ability of the 2465 protein to bind to a 2465 target molecule by one

of the methods described above for determining direct binding. Determining the ability of the 2465 protein to bind to a 2465 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In another embodiment, determining the ability of the test compound to modulate the activity of a 2465 protein can be accomplished by determining the ability of the 2465 protein to further modulate the activity of a downstream effector of a 2465 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 2465 protein or biologically active portion thereof with a known compound which binds the 2465 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 2465 protein, wherein determining the ability of the test compound to interact with the 2465 protein comprises determining the ability of the 2465 protein to preferentially bind to or modulate the activity of a 2465 target molecule.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either 2465 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 2465 protein, or interaction of a 2465 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ 2465 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads

(Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 2465 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 2465 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a 2465 protein or a 2465 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 2465 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with 2465 protein or target molecules but which do not interfere with binding of the 2465 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or 2465 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 2465 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 2465 protein or target molecule.

In another embodiment, modulators of 2465 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of 2465 mRNA or protein in the cell is determined. The level of expression of 2465 mRNA or protein in the presence of the candidate compound is compared to the level of expression of 2465 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of 2465 expression based on this comparison. For example, when expression of 2465 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 2465 mRNA or protein

expression. Alternatively, when expression of 2465 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 2465 mRNA or protein expression. The level of 2465 mRNA or protein expression in the cells can be determined by methods described herein for detecting 2465 mRNA or protein.

In yet another aspect of the invention, the 2465 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 2465 ("2465-binding proteins" or "2465-bp") and are involved in 2465 activity. Such 2465-binding proteins are also likely to be involved in the propagation of signals by the 2465 proteins or 2465 targets as, for example, downstream elements of a 2465-mediated signaling pathway. Alternatively, such 2465-binding proteins are likely to be 2465 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 2465 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a 2465-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 2465 protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a 2465

protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for hepatic, bone, or cardiovascular disorders, as described herein.

Examples of animal models of hepatic fibrosis include animal models suffering from carbon tetrachloride intoxication, iron and alcohol intoxication, streptococcal cell wall administration, and bile duct ligation, *e.g.*, in rats, as well as mice suffering from schistosomiasis. These animal models are known in the art and are described in, for example, Czaja *et al.* (1989) *J. Cell. Biol.* 108:2477-2482; Manthey *et al.* (1990) *Growth Factors* 4:17-26; Bissell *et al.* (1995) *J. Clin. Invest.* 96:447-455; Tsukamoto *et al.* (1995) *J. Clin. Invest.* 96:620-630; Alcolado *et al.* (1997) *Clin. Sci.* 92:103-112; Cales (1998) *Biomed. and Pharmacother.* 52:259-263.

Animal-based model systems of cardiovascular disease may include, but are not limited to, non-recombinant and engineered transgenic animals.

Non-recombinant animal models for cardiovascular disease may include, for example, genetic models. Such genetic cardiovascular disease models may include, for example, apoB or apoR deficient pigs (Rapacz, *et al.*, 1986, *Science* 234:1573-1577) and Watanabe heritable hyperlipidemic (WHHL) rabbits (Kita *et al.*, 1987, *Proc. Natl. Acad. Sci USA* 84: 5928-5931). Transgenic mouse models in cardiovascular disease and angiogenesis are reviewed in Carmeliet, P. and Collen, D. (2000) J. Pathol. 190:387-405.

Non-recombinant, non-genetic animal models of atherosclerosis may include, for example, pig, rabbit, or rat models in which the animal has been exposed to either chemical wounding through dietary supplementation of LDL, or mechanical wounding through balloon catheter angioplasty. Animal models of cardiovascular disease also include rat myocardial infarction models (described in, for example, Schwarz, ER *et al.* (2000) *J. Am. Coll. Cardiol.* 35:1323-1330) and models of chromic cardiac ischemia in rabbits (described in, for example, Operschall, C *et al.* (2000) *J. Appl. Physiol.* 88:1438-1445).

Models for studying angiogenesis *in vivo* include tumor cell-induced angiogenesis and tumor metastasis (Hoffman, RM (1998-99) *Cancer Metastasis Rev.* 17:271-277; Holash, J *et al.* (1999) *Oncogene* 18:5356-5362; Li, CY *et al.* (2000) *J. Natl Cancer Inst.* 92:143-147), matrix induced angiogenesis (US Patent No. 5,382,514), the disc angiogenesis system (Kowalski, J. *et al.* (1992) *Exp. Mol. Pathol.* 56:1-19), the

rodent mesenteric-window angiogenesis assay (Norrby, K (1992) *EXS* 61:282-286), experimental choroidal neovascularization in the rat (Shen, WY *et al.* (1998) *Br. J. Ophthalmol.* 82:1063-1071), and the chick embryo development (Brooks, PC *et al. Methods Mol. Biol.* (1999) 129:257-269) and chick embryo chorioallantoic membrane (CAM) models (McNatt LG *et al.* (1999) *J. Ocul. Pharmacol. Ther.* 15:413-423; Ribatti, D *et al.* (1996) *Int. J. Dev. Biol.* 40:1189-1197), and are reviewed in Ribatti, D and Vacca, A (1999) *Int. J. Biol. Markers* 14:207-213.

Models for studying vascular tone *in vivo* include the rabbit femoral artery model (Luo *et al.* (2000) *J. Clin. Invest.* 106:493-499), eNOS knockout mice (Hannan *et al.* (2000) *J. Surg. Res.* 93:127-132), rat models of cerebral ischemia (Cipolla *et al.* (2000) *Stroke* 31:940-945), the renin-angiotensin mouse system (Cvetkovik *et al.* (2000) *Kidney Int.* 57:863-874), the rat lung transplant model (Suda *et al.* (2000) *J. Thorac. Cardiovasc. Surg.* 119:297-304), the New Zeland White rabbit model of intracranial hypertension (Richards *et al.* (1999) *Acta Neurochir.* 141:1221-1227), the spontaneously hypertensive (SH) rat neurogenic model of chronic hypertension (Stekiel *et al.* (1999) *Anesthesiology* 91:207-214), the Prague hypertensive rat (PHR) (Vogel *et al.* (1999) *Clin. Sci.* 97:91-98), chronically angiotensin II (Ang II)-infused rats (Pasquie *et al.* (1999) *Hypertension* 33:830-834), Dahl-salt-sensitive rats (Boulanger (1999) *J. Mol. Cell. Cardiol.* 31:39-49), the mouse model of arterial remodeling (Bryant *et al.* (1999) *Circ. Res.* 84:323-328), and the obese Zucker (fa/fa) rat (Golub *et al.* (1998) *Hypertens. Res.* 21:283-288).

Cells that contain and express 2465 gene sequences which encode a 2465 protein, and, further, exhibit cellular phenotypes associated with cardiovascular disease, may be used to identify compounds that exhibit anti-cardiovascular disease activity. Such cells may include non-recombinant monocyte cell lines, such as U937 (ATCC # CRL-1593), THP-1 (ATCC #TIB-202), and P388D1 (ATCC # TIB-63); endothelial cells such as human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVEC), and bovine aortic endothelial cells (BAECs); as well as generic mammalian cell lines such as HeLa cells and COS cells, *e.g.*, COS-7 (ATCC # CRL-1651). Further, such cells may include recombinant, transgenic cell lines. For example, the cardiovascular disease animal models of the invention, discussed above, may be used to generate cell lines, containing one or more cell types involved in

cardiovascular disease, that can be used as cell culture models for this disorder. While primary cultures derived from the cardiovascular disease transgenic animals of the invention may be utilized, the generation of continuous cell lines is preferred. For examples of techniques which may be used to derive a continuous cell line from the transgenic animals, see Small *et al*., (1985) *Mol. Cell Biol.* 5:642-648.

Alternatively, cells of a cell type known to be involved in cardiovascular disease may be transfected with sequences capable of increasing or decreasing the amount of 2465 gene expression within the cell. For example, 2465 gene sequences may be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous 2465 gene sequences are present, they may be either overexpressed or, alternatively disrupted in order to underexpress or inactivate 2465 gene expression.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a 2465 modulating agent, an antisense 2465 nucleic acid molecule, a 2465-specific antibody, or a 2465-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

Any of the compounds, including but not limited to compounds such as those identified in the foregoing assay systems, may be tested for the ability to ameliorate hepatic, bone, or cardiovascular disorder symptoms. Cell-based and animal model-based assays for the identification of compounds exhibiting such an ability to ameliorate hepatic, bone, or cardiovascular disorder systems are described herein.

In one aspect, cell-based systems, as described herein, may be used to identify compounds which may act to ameliorate hepatic, bone, or cardiovascular disorder symptoms. For example, such cell systems may be exposed to a compound, suspected of exhibiting an ability to ameliorate hepatic, bone, or cardiovascular disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of hepatic, bone, or cardiovascular disorder symptoms in the exposed cells.

After exposure, the cells are examined to determine whether one or more of the hepatic, bone, or cardiovascular disorder cellular phenotypes has been altered to resemble a more normal or more wild type, non-hepatic or non-bone associated disease phenotype. Cellular phenotypes that are associated with hepatic, bone, or cardiovascular disorder states include aberrant proliferation and migration, deposition of extracellular matrix components, and expression of growth factors, cytokines, and other inflammatory mediators.

In addition, animal-based hepatic, bone, or cardiovascular disorder or disease systems, such as those described herein, may be used to identify compounds capable of ameliorating hepatic, bone, or cardiovascular disorder symptoms. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies, and interventions which may be effective in treating hepatic, bone, or cardiovascular disorders. For example, animal models may be exposed to a compound, suspected of exhibiting an ability to ameliorate hepatic, bone, or cardiovascular disorder symptoms, at a sufficient concentration and for a time sufficient to elicit such an amelioration of hepatic, bone, or cardiovascular disorder symptoms in the exposed animals. The response of the animals to the exposure may be monitored by assessing the reversal of disorders associated with hepatic, bone, or cardiovascular disorders, for example, by measuring liver loss and/or measuring bone loss before and after treatment.

With regard to intervention, any treatments which reverse any aspect of hepatic, bone, or cardiovascular disorder symptoms should be considered as candidates for human hepatic, bone, or cardiovascular disorder therapeutic intervention. Dosages of test agents may be
determined by deriving dose-response curves.

Additionally, gene expression patterns may be utilized to assess the ability of a compound to ameliorate hepatic, bone, or cardiovascular disorder symptoms. For example, the expression pattern of one or more genes may form part of a "gene expression profile" or “transcriptional profile” which may be then be used in such an assessment. "Gene expression profile" or “transcriptional profile”, as used herein, includes the pattern of mRNA expression obtained for a given tissue or cell type under a given set of conditions. Such conditions may include, but are not limited to, atherosclerosis, ischemia/reperfusion, hypertension, restenosis, arterial inflammation,

and liver fibrosis, including any of the control or experimental conditions described herein. Gene expression profiles may be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR. In one embodiment, 2465 gene sequences may be used as probes and/or PCR primers for the generation and corroboration of such gene expression profiles.

Gene expression profiles may be characterized for known states, either hepatic, bone, or cardiovascular disorders or normal, within the cell- and/or animal-based model systems. Subsequently, these known gene expression profiles may be compared to ascertain the effect a test compound has to modify such gene expression profiles, and to cause the profile to more closely resemble that of a more desirable profile.

For example, administration of a compound may cause the gene expression profile of a hepatic, bone, or cardiovascular disorder model system to more closely resemble the control system. Administration of a compound may, alternatively, cause the gene expression profile of a control system to begin to mimic a hepatic, bone, or cardiovascular disorder state. Such a compound may, for example, be used in further characterizing the compound of interest, or may be used in the generation of additional animal models.

2. <u>Predictive Medicine</u>

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 2465 protein and/or nucleic acid expression as well as 2465 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a hepatic, bone, or cardiovascular disorder, associated with aberrant or unwanted 2465 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with 2465 protein, nucleic acid expression or activity. For example, mutations in a 2465 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a

disorder characterized by or associated with 2465 protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of 2465 in clinical trials.

These and other agents are described in further detail in the following sections.

A. Diagnostic Assays

The present invention encompasses methods for diagnostic and prognostic evaluation of hepatic, bone, or cardiovascular disorder conditions, and for the identification of subjects exhibiting a predisposition to such conditions.

An exemplary method for detecting the presence or absence of 2465 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 2465 protein or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes 2465 protein such that the presence of 2465 protein or nucleic acid is detected in the biological sample. A preferred agent for detecting 2465 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 2465 mRNA or genomic DNA. The nucleic acid probe can be, for example, the 2465 nucleic acid set forth in SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 15, 20, 25, 30, 35, 40, 45, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 2465 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting 2465 protein is an antibody capable of binding to 2465 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term

"biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect 2465 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 2465 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of 2465 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of 2465 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of 2465 protein include introducing into a subject a labeled anti-2465 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 2465 protein, mRNA, or genomic DNA, such that the presence of 2465 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 2465 protein, mRNA or genomic DNA in the control sample with the presence of 2465 protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of 2465 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting 2465 protein or mRNA in a biological sample; means for determining the amount of 2465 in the sample; and means for comparing the amount of 2465 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 2465 protein or nucleic acid.

B. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a hepatic, bone, or cardiovascular disease or disorder associated with aberrant or unwanted 2465 expression or activity. As used herein, the term "aberrant" includes a 2465 expression or activity which deviates from the wild type 2465 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 2465 expression or activity is intended to include the cases in which a mutation in the 2465 gene causes the 2465 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional 2465 protein or a protein which does not function in a wild-type fashion, *e.g.*, a protein which does not interact with a 2465 ligand or substrate, or one which interacts with a non-2465 ligand or substrate. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as cellular proliferation. For example, the term unwanted includes a 2465 expression pattern or a 2465 protein activity which is undesirable in a subject.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in 2465 protein activity or nucleic acid expression, such as a hepatic, bone, or cardiovascular disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a hepatic, bone, or cardiovascular disorder associated with a misregulation in 2465 protein activity or nucleic acid expression. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted 2465 expression or activity in which a test sample is obtained from a subject and 2465 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of 2465 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 2465 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 2465 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a hepatic, bone, or cardiovascular disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a hepatic, bone, or cardiovascular disorder associated with aberrant or unwanted 2465 expression or activity in which a test sample is obtained and 2465 protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of 2465 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted 2465 expression or activity).

The methods of the invention can also be used to detect genetic alterations in a 2465 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 2465 protein activity or nucleic acid expression, such as a proliferative disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 2465-protein, or the mis-expression of the 2465 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 2465 gene; 2) an addition of one or more nucleotides to a 2465 gene; 3) a substitution of one or more nucleotides of a 2465 gene, 4) a chromosomal rearrangement of a 2465 gene; 5) an alteration in the level of a messenger RNA transcript of a 2465 gene, 6) aberrant modification of a 2465 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 2465 gene, 8) a non-wild type level of a 2465-protein, 9) allelic loss of a 2465 gene, and 10) inappropriate post-translational modification of a 2465-protein. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in a 2465 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the 2465-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 2465 gene under conditions such that hybridization and amplification of the 2465-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Other amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a 2465 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 2465 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 2465 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 2465 gene and detect mutations by comparing the sequence of the sample 2465 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the 2465 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type 2465 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex

such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 2465 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a 2465 sequence, *e.g.*, a wild-type 2465 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like (described in, for example, U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 2465 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 2465 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary

structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation

will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 2465 gene.

Furthermore, any cell type or tissue in which 2465 is expressed may be utilized in the prognostic assays described herein.

C. Monitoring of Effects During Clinical Trials

The present invention provides methods for evaluating the efficacy of drugs and monitoring the progress of patients involved in clinical trials for the treatment of hepatic, bone, or cardiovascular disorders.

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a 2465 protein (*e.g.*, the modulation of cell proliferation and/or migration) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 2465 gene expression, protein levels, or upregulate 2465 activity, can be monitored in clinical trials of subjects exhibiting decreased 2465 gene expression, protein levels, or downregulated 2465 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 2465 gene expression, protein levels, or downregulate 2465 activity, can be monitored in clinical trials of subjects exhibiting increased 2465 gene expression, protein levels, or upregulated 2465 activity. In such clinical trials, the expression or activity of a 2465 gene, and preferably, other genes that have been implicated in, for example, a 2465-associated disorder can be used as a "read out" or markers of the phenotype a particular cell, *e.g.*, an endothelial cell. In addition, the expression of a 2465 gene, or the level of 2465 protein activity may be used as a read out of a particular drug or agent's effect on a hepatic, bone, or cardiovascular disorders state.

For example, and not by way of limitation, genes, including 2465, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates 2465 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on 2465-associated disorders (*e.g.*, hepatic, bone, or cardiovascular disorders characterized by deregulated endothelial cell activity), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of 2465 and other genes implicated in the 2465-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of 2465 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 2465 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 2465 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the 2465 protein, mRNA, or genomic DNA in the pre-administration sample with the 2465 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of 2465 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of 2465 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an

embodiment, 2465 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

3. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 2465 expression or activity, *e.g.* a hepatic, bone, or cardiovascular disorder. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 2465 molecules of the present invention or 2465 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

Treatment is defined as the application or administration of a therapeutic agent to a patient, or the application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease, the symptoms of disease or the predisposition toward disease as described herein.

A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

A. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a hepatic, bone, or cardiovascular disorder or condition associated with an aberrant or unwanted 2465 expression or activity, by administering to the subject a 2465 or an agent which modulates 2465 expression or at least one 2465 activity. Subjects at risk for a hepatic, bone, or cardiovascular disorder which is caused or contributed to by aberrant or unwanted 2465 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 2465 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 2465 aberrancy, for example, a 2465, 2465 agonist or 2465 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

B. Therapeutic Methods

Described herein are methods and compositions whereby hepatic, bone, or cardiovascular disorder symptoms may be ameliorated. Certain hepatic, bone, or cardiovascular disorders are brought about, at least in part, by an excessive level of a gene product, or by the presence of a gene product exhibiting an abnormal or excessive activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of hepatic, bone, or cardiovascular disorder symptoms. Techniques for the reduction of gene expression levels or the activity of a protein are discussed below.

Alternatively, certain other hepatic, bone, or cardiovascular disorders are brought about, at least in part, by the absence or reduction of the level of gene expression, or a reduction in the level of a protein's activity. As such, an increase in the level of gene expression and/or the activity of such proteins would bring about the amelioration of hepatic, bone, or cardiovascular disorder symptoms.

In some cases, the up-regulation of a gene in a disease state reflects a protective role for that gene product in responding to the disease condition. Enhancement of such a gene's expression, or the activity of the gene product, will reinforce the protective effect it exerts. Some hepatic, bone, or cardiovascular disorder states may result from an

abnormally low level of activity of such a protective gene. In these cases also, an increase in the level of gene expression and/or the activity of such gene products would bring about the amelioration of hepatic, bone, or cardiovascular disorder symptoms. Techniques for increasing target gene expression levels or target gene product activity levels are discussed herein.

Accordingly, another aspect of the invention pertains to methods of modulating 2465 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 2465 or agent that modulates one or more of the activities of 2465 protein activity associated with the cell (*e.g.,* a hepatic cell). An agent that modulates 2465 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 2465 protein (*e.g.*, a 2465 ligand or substrate), a 2465 antibody, a 2465 agonist or antagonist, a peptidomimetic of a 2465 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 2465 activities. Examples of such stimulatory agents include active 2465 protein and a nucleic acid molecule encoding 2465 that has been introduced into the cell. In another embodiment, the agent inhibits one or more 2465 activities. Examples of such inhibitory agents include antisense 2465 nucleic acid molecules, anti-2465 antibodies, and 2465 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 2465 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 2465 expression or activity. In another embodiment, the method involves administering a 2465 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 2465 expression or activity.

Stimulation of 2465 activity is desirable in situations in which 2465 is abnormally downregulated and/or in which increased 2465 activity is likely to have a beneficial effect. Likewise, inhibition of 2465 activity is desirable in situations in which

2465 is abnormally upregulated and/or in which decreased 2465 activity is likely to have a beneficial effect.

(i) Methods for Inhibiting Target Gene Expression, Synthesis, or Activity

As discussed above, genes involved in hepatic, bone, or cardiovascular disorders may cause such disorders via an increased level of gene activity. In some cases, such up-regulation may have a causative or exacerbating effect on the disease state. A variety of techniques may be used to inhibit the expression, synthesis, or activity of such genes and/or proteins.

For example, compounds such as those identified through assays described above, which exhibit inhibitory activity, may be used in accordance with the invention to ameliorate hepatic, bone, or cardiovascular disorder symptoms. Such molecules may include, but are not limited to, small organic molecules, peptides, antibodies, and the like.

For example, compounds can be administered that compete with endogenous ligand for the 2465 protein. The resulting reduction in the amount of ligand-bound 2465 protein will modulate endothelial cell physiology. Compounds that can be particularly useful for this purpose include, for example, soluble proteins or peptides, such as peptides comprising one or more of the extracellular domains, or portions and/or analogs thereof, of the 2465 protein, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins. (For a discussion of the production of Ig-tailed fusion proteins, see, for example, U.S. Pat. No. 5,116,964). Alternatively, compounds, such as ligand analogs or antibodies, that bind to the 2465 receptor site, but do not activate the protein, (*e.g.*, receptor-ligand antagonists) can be effective in inhibiting 2465 protein activity.

Further, antisense and ribozyme molecules which inhibit expression of the 2465 gene may also be used in accordance with the invention to inhibit aberrant 2465 gene activity. Still further, triple helix molecules may be utilized in inhibiting aberrant 2465 gene activity.

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 2465 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by

conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave 2465 mRNA transcripts to thereby inhibit translation of 2465 mRNA. A ribozyme having specificity for a 2465-encoding nucleic acid can be designed based upon the nucleotide sequence of a 2465 cDNA disclosed herein (*i.e.*, SEQ ID NO:1). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 2465-encoding mRNA (see, for example, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742).

Alternatively, 2465 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see, for example, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418).

2465 gene expression can also be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 2465 (*e.g.*, the 2465 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 2465 gene in target cells (see, for example, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15).

Antibodies that are both specific for the 2465 protein and interfere with its activity may also be used to modulate or inhibit 2465 protein function. Such antibodies may be generated using standard techniques described herein, against the 2465 protein itself or against peptides corresponding to portions of the protein. Such antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, or chimeric antibodies.

In instances where the target gene protein is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin liposomes may be used to deliver the antibody or a fragment of the Fab region which binds to the target epitope into cells. Where fragments of the antibody are used, the smallest inhibitory fragment which binds to the target protein's binding domain is preferred. For example, peptides having an amino acid sequence corresponding to the domain of the variable region of the antibody that binds to the target gene protein may be used. Such peptides may be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (described in, for example, Creighton (1983), *supra*; and Sambrook *et al.* (1989) *supra*). Single chain neutralizing antibodies which bind to intracellular target gene epitopes may also be administered. Such single chain antibodies may be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population by utilizing, for example, techniques such as those described in Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

In some instances, the target gene protein is extracellular, or is a transmembrane protein, such as the 2465 protein. Antibodies that are specific for one or more extracellular domains of the 2465 protein, for example, and that interfere with its activity, are particularly useful in treating hepatic, bone, or cardiovascular disorders. Such antibodies are especially efficient because they can access the target domains directly from the bloodstream. Any of the administration techniques described below which are appropriate for peptide administration may be utilized to effectively administer inhibitory target gene antibodies to their site of action.

(ii) Methods for Restoring or Enhancing Target Gene Activity

Genes that cause hepatic, bone, or cardiovascular disorders may be underexpressed within hepatic, bone, or cardiovascular disorder situations. Alternatively, the activity of the protein products of such genes may be decreased, leading to the development of hepatic, bone, or cardiovascular disorder symptoms. Such down-regulation of gene expression or decrease of protein activity might have a causative or exacerbating effect on the disease state.

In some cases, genes that are up-regulated in the disease state might be exerting a protective effect. Specifically, 2465 is up-regulated in stellate cells (the main effectors of liver fibrosis), Furthermore, 2465 is up-regulated during osteoblast differentiation. 2465 is also up-regulated during laminar shear stress, proliferation, and in the presence of IL-1β (stimuli relevant to angiogenesis, atherosclerosis, and vascular tone). A variety of techniques may be used to decrease the expression, synthesis, or activity of 2465 genes and/or proteins that exert a causatory effect on hepatic, bone, or cardiovascular disorder conditions.

Described in this section are methods whereby the level 2465 activity may be modulated to levels wherein hepatic, bone, or cardiovascular disorder symptoms are ameliorated. The level of 2465 activity may be modulated, for example, by either modulating the level of 2465 gene expression or by modulating the level of active 2465 protein which is present.

For example, an inhibitor of a 2465 protein, at a level sufficient to ameliorate hepatic, bone, or cardiovascular disorder symptoms may be administered to a patient exhibiting such symptoms. Any of the techniques discussed below may be used for such

administration. One of skill in the art will readily know how to determine the concentration of effective, non-toxic doses of an inhibitor of the 2465 protein, utilizing techniques such as those described below.

Additionally, antisense 2465 DNA sequences may be directly administered to a patient exhibiting hepatic, bone, or cardiovascular disorder symptoms, at a concentration sufficient to reduce the level of 2465 protein such that hepatic, bone, or cardiovascular disorder symptoms are ameliorated. Any of the techniques discussed below, which achieve intracellular administration of compounds, such as, for example, liposome administration, may be used for the administration of such antisense DNA molecules. The DNA molecules may be produced, for example, by recombinant techniques such as those described herein.

Further, subjects may be treated by gene replacement therapy. One or more copies of an antagonist of the 2465 molecule, *e.g.*, a portion of the 2465 gene, may be inserted into cells using vectors which include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes. Additionally, techniques such as those described above may be used for the introduction of 2465 gene sequences into human cells.

Cells, preferably, autologous cells, containing 2465 antagonist expressing gene sequences may then be introduced or reintroduced into the subject at positions which allow for the amelioration of hepatic, bone, or cardiovascular disorder symptoms. Such cell replacement techniques may be preferred, for example, when the gene product is a secreted, extracellular gene product.

C. Pharmacogenomics

The 2465 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 2465 activity (*e.g.*, 2465 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 2465-associated disorders (*e.g.*, hepatic, bone, or cardiovascular disorders) associated with aberrant or unwanted 2465 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can

lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 2465 molecule or a 2465 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 2465 molecule or 2465 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-

associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, a 2465 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 2465 molecule or 2465 modulator of the present

invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 2465 molecule or 2465 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

4. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

A. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the 2465 nucleotide sequences, described herein, can be used to map the location of the 2465 genes on a chromosome. The mapping of the 2465 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease. The 2465 gene has been mapped to human chromosome position 15q14-15.

Briefly, 2465 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 2465 nucleotide sequences. Computer analysis of the 2465 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human

chromosomes. [redacted] those hybrids containing the human [redacted]corresponding to the 2465 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the 2465 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a 2465 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity

for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al*: (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 2465 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

B. Tissue Typing

The 2465 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is

digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 2465 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The 2465 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of 2465 gene sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:1 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 2465 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database,

positive identification of the individual, living or dead, can be made from extremely small tissue samples.

C. Use of Partial 2465 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of 2465 gene sequences are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the 2465 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions having a length of at least 20 bases, preferably at least 30 bases.

The 2465 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 2465 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, 2465 primers/probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

D. Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising 2465 sequence information is also provided. As used herein, "2465 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the 2465 molecules of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information "related to" said 2465 sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantitative detection); detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon 2465 sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the 2465 sequence information.

A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the 2465 sequence information.

By providing 2465 sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder, wherein the method comprises the steps of determining 2465 sequence information associated with the subject and based on the 2465 sequence information, determining whether the subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 2465-associated disease or disorder or a pre-disposition to a disease associated with a 2465 wherein the method comprises the steps of determining 2465 sequence information associated with the subject, and based on the 2465 sequence information, determining whether the subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated

disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder associated with 2465, said method comprising the steps of receiving 2465 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 2465 and/or a 2465-associated disease or disorder, and based on one or more of the phenotypic information, the 2465 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder, said method comprising the steps of receiving information related to 2465 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 2465 and/or related to a 2465-associated disease or disorder, and based on one or more of the phenotypic information, the 2465 information, and the acquired information, determining whether the subject has a 2465-associated disease or disorder or a pre-disposition to a 2465-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention also includes an array comprising a 2465 sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be 2465. This allows a

profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a 2465-associated disease or disorder, progression of 2465-associated disease or disorder, and processes, such a cellular transformation associated with the 2465-associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of 2465 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including 2465) that could serve as a molecular target for diagnosis or therapeutic intervention.

5. Recombinant Expression Vectors and Host Cells

The methods of the invention include the use of vectors, preferably expression vectors, containing a nucleic acid encoding a 2465 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the methods of the invention may include other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185,

Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, 2465 proteins, mutant forms of 2465 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 2465 proteins in prokaryotic or eukaryotic cells, *e.g.*,. for use in the cell-based assays of the invention. For example, 2465 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA)

and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in 2465 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for 2465 proteins, for example. In a preferred embodiment, a 2465 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the 2465 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-

943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, 2465 proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters

(Kessel and Gru[illegible]990) *Science* 249:374-379) and the α-[illegible]rotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The expression characteristics of an endogenous 2465 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous 2465 gene. For example, an endogenous 2465 gene which is normally "transcriptionally silent", *i.e.*, a 2465 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous 2465 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous 2465 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

The methods of the invention further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 2465 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*,

Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to the use of host cells into which a 2465 nucleic acid molecule of the invention is introduced, *e.g.*, a 2465 nucleic acid molecule within a recombinant expression vector or a 2465 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 2465 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO), COS cells, or human umbilical vein endothelial cells (HUVEC)). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418,

hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a 2465 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 2465 protein. Accordingly, the invention further provides methods for producing a 2465 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a 2465 protein has been introduced) in a suitable medium such that a 2465 protein is produced. In another embodiment, the method further comprises isolating a 2465 protein from the medium or the host cell.

6. Cell- and Animal-Based Model Systems

Described herein are cell- and animal-based systems which act as models for hepatic, bone, or cardiovascular disorders. These systems may be used in a variety of applications. For example, the cell- and animal-based model systems may be used to further characterize differentially expressed genes associated with hepatic, bone, or cardiovascular disorders, *e.g.*, 2465. In addition, animal- and cell-based assays may be used as part of screening strategies designed to identify compounds which are capable of ameliorating hepatic, bone, or cardiovascular disorder symptoms, as described, below. Thus, the animal- and cell-based models may be used to identify drugs, pharmaceuticals, therapies and interventions which may be effective in treating hepatic, bone, or cardiovascular disorders. Furthermore, such animal models may be used to determine the LD50 and the ED50 in animal subjects, and such data can be used to determine the *in vivo* efficacy of potential hepatic, bone, or cardiovascular disorder treatments.

A. Animal-Based Systems

Animal-based model systems of hepatic, bone, or cardiovascular disorders may include, but are not limited to, non-recombinant and engineered transgenic animals.

Non-recombinant animal models for hepatic, bone, or cardiovascular disorders may include, for example, genetic models.

Additionally, animal models exhibiting hepatic, bone, or cardiovascular disorders symptoms may be engineered by using, for example, 2465 gene sequences described above, in conjunction with techniques for producing transgenic animals that are well known to those of skill in the art. For example, 2465 gene sequences may be introduced into, and overexpressed in, the genome of the animal of interest, or, if endogenous 2465 gene sequences are present, they may either be overexpressed or, alternatively, be disrupted in order to underexpress or inactivate 2465 gene expression.

Non-recombinant animal models for cardiovascular disorders are described *supra*.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which 2465-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous 2465 sequences have been introduced into their genome or homologous recombinant animals in which endogenous 2465 sequences have been altered. Such animals are useful for studying the function and/or activity of a 2465 and for identifying and/or evaluating modulators of 2465 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous 2465 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal for use in the methods of the invention can be created by introducing a 2465-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The 2465 cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human 2465 gene, such as a mouse or rat 2465 gene, can be used as a transgene. Alternatively, a 2465 gene homologue, such as another 2465 family member, can be isolated based on hybridization to the 2465 cDNA sequences of SEQ ID NO:1 and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a 2465 transgene to direct expression of a 2465 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a 2465 transgene in its genome and/or expression of 2465 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 2465 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a 2465 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the 2465 gene. The 2465 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1), but more preferably, is a non-human homologue of a human 2465 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1). For example, a mouse 2465 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous 2465 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed

such that, upon homologous recombination, the endogenous 2465 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous 2465 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous 2465 protein). In the homologous recombination nucleic acid molecule, the altered portion of the 2465 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the 2465 gene to allow for homologous recombination to occur between the exogenous 2465 gene carried by the homologous recombination nucleic acid molecule and an endogenous 2465 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking 2465 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced 2465 gene has homologously recombined with the endogenous 2465 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_O phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

The 2465 transgenic animals that express 2465 mRNA or a 2465 peptide (detected immunocytochemically, using antibodies directed against 2465 epitopes) at easily detectable levels should then be further evaluated to identify those animals which display characteristic hepatic, bone, or cardiovascular disorder symptoms. Such symptoms may include, for example, increased prevalence and size of fatty streaks and/or hepatic, bone, or cardiovascular disorder plaques.

Additionally, specific cell types within the transgenic animals may be analyzed and assayed for cellular phenotypes characteristic of hepatic, bone, or cardiovascular disorders. In the case of monocytes, such phenotypes may include but are not limited to increases in rates of LDL uptake, adhesion to endothelial cells, transmigration, foam cell

formation, fatty streak formation, and production of foam cell specific products. Cellular phenotypes may include a particular cell type's pattern of expression of genes associated with hepatic, bone, or cardiovascular disorders as compared to known expression profiles of the particular cell type in animals exhibiting hepatic, bone, or cardiovascular disorder symptoms.

B. Cell-Based Systems

Cells that contain and express 2465 gene sequences which encode a 2465 protein, and, further, exhibit cellular phenotypes associated with hepatic, bone, or cardiovascular disorders, may be used to identify compounds that exhibit anti-hepatic, bone, or cardiovascular disorder activity. Such cells may include non-recombinant monocyte cell lines, such as U937 (ATCC# CRL-1593), THP-1 (ATCC#TIB-202), and P388D1 (ATCC# TIB-63); endothelial cells such as human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVEC), and bovine aortic endothelial cells (BAECs); hepatic cells such as human Hepa; as well as generic mammalian cell lines such as HeLa cells and COS cells, *e.g.*, COS-7 (ATCC# CRL-1651). Further, such cells may include recombinant, transgenic cell lines. For example, the hepatic, bone, or cardiovascular disorder animal models of the invention, discussed above, may be used to generate cell lines, containing one or more cell types involved in hepatic, bone, or cardiovascular disorders, that can be used as cell culture models for this disorder. While primary cultures derived from the hepatic, bone, or cardiovascular disorder transgenic animals of the invention may be utilized, the generation of continuous cell lines is preferred. For examples of techniques which may be used to derive a continuous cell line from the transgenic animals, see Small *et al.*, (1985) *Mol. Cell Biol.* 5:642-648.

Alternatively, cells of a cell type known to be involved in hepatic, bone, or cardiovascular disorders may be transfected with sequences capable of increasing or decreasing the amount of 2465 gene expression within the cell. For example, 2465 gene sequences may be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous 2465 gene sequences are present, they may be either overexpressed or, alternatively disrupted in order to underexpress or inactivate 2465 gene expression.

In order to overexpress a 2465gene, the coding portion of the 2465 gene may be ligated to a regulatory sequence which is capable of driving gene expression in the cell type of interest, *e.g.*, an endothelial cell. Such regulatory regions will be well known to those of skill in the art, and may be utilized in the absence of undue experimentation. Recombinant methods for expressing target genes are described above.

For underexpression of an endogenous 2465 gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the cell type of interest, the endogenous 2465 alleles will be inactivated. Preferably, the engineered 2465 sequence is introduced via gene targeting such that the endogenous 2465 sequence is disrupted upon integration of the engineered 2465 sequence into the cell's genome. Transfection of host cells with 2465 genes is discussed, above.

Cells treated with compounds or transfected with 2465 genes can be examined for phenotypes associated with hepatic, bone, or cardiovascular disorders. In the case of hepatocytes, such phenotypes include but are not limited to overproduction of matrix components. In the case of osteocytes, such phenotypes include but are not limited to expression of cytokines or growth factors. Expression of cytokines or growth factors may be measured using any of the assays described herein.

Similarly, hepatic, bone, or cardiovascular cells can be treated with test compounds or transfected with genetically engineered 2465genes. The hepatic, bone, or cardiovascular cells can then be examined for phenotypes associated with hepatic, bone, or cardiovascular disorders, including, but not limited to changes in cellular morphology, cell proliferation, and cell migration; or for the effects on production of other proteins involved in hepatic, bone, or cardiovascular disorders such as adhesion molecules (*e.g.*, ICAM, VCAM), PDGF, and E-selectin.

Transfection of 2465 nucleic acid may be accomplished by using standard techniques (described in, for example, Ausubel (1989) *supra*). Transfected cells should be evaluated for the presence of the recombinant 2465 gene sequences, for expression and accumulation of 2465 mRNA, and for the presence of recombinant 2465 protein production. In instances wherein a decrease in 2465 gene expression is desired, standard techniques may be used to demonstrate whether a decrease in endogenous 2465 gene expression and/or in 2465 protein production is achieved.

7. Pharmaceutical Compositions

Active compounds for use in the methods of the invention can be incorporated into pharmaceutical compositions suitable for administration. As used herein, the language "active compounds" includes 2465 nucleic acid molecules, fragments of 2465 proteins, and anti-2465 antibodies, as well as identified compounds that modulate 2465 gene expression, synthesis, and/or activity. Such compositions typically comprise the compound, nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For

intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a 2465 protein or a 2465 ligand) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is

applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically

acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals. In one embodiment, a therapeutically effective dose refers to that amount of an active compound sufficient to result in amelioration of symptoms of hepatic, bone, or cardiovascular disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as

determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses the identification and/or use of agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is

understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites

(*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil . decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

8. Isolated Nucleic Acid Molecules

The nucleotide sequence of the isolated human 2465 cDNA and the predicted amino acid sequence of the human 2465 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. The nucleotide sequence encoding human 2465 is identical to the nucleic acid molecule with GenBank Accession Number D38449 (Hata *et al. BBA* (1995) 1261:121-125).

The human 2465 gene, which is approximately 2816 nucleotides in length, encodes a protein having a molecular weight of approximately 59.34 kD and which is approximately 516 amino acid residues in length.

The methods of the invention include the use of isolated nucleic acid molecules that encode 2465 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 2465-encoding nucleic acid molecules (*e.g.*, 2465 mRNA) and fragments for use as PCR primers for the amplification or mutation of 2465 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated 2465 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, as a hybridization probe, 2465 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to 2465 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human 2465 cDNA. This cDNA comprises sequences encoding the human 2465 protein (*i.e.*, "the coding region").

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, or a portion of any of this nucleotide sequence. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, thereby forming a stable duplex.

In still another preferred embodiment, the methods of the invention include the use of an isolated nucleic acid molecule that comprises a nucleotide sequence which is at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, or a portion of any of this nucleotide sequence.

Moreover, the methods of the invention include the use of a nucleic acid molecule that comprises only a portion of the nucleic acid sequence of SEQ ID NO:1, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 2465 protein, *e.g.*, a biologically active portion of a 2465 protein. The nucleotide sequence determined from the cloning of the 2465 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 2465 family members, as well as 2465 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, of an anti-sense sequence of SEQ ID NO:1, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1. In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, or more

nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1.

Probes based on the 2465 nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a 2465 protein, such as by measuring a level of a 2465-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting 2465 mRNA levels or determining whether a genomic 2465 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a 2465 protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 which encodes a polypeptide having a 2465 biological activity (the biological activities of the 2465 protein is described herein), expressing the encoded portion of the 2465 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 2465 protein.

The methods of the invention further encompass nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, due to degeneracy of the genetic code and thus encode the same 2465 protein as those encoded by the nucleotide sequence shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

In addition to the 2465 nucleotide sequence shown in SEQ ID NO:1, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the 2465 protein may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the 2465 gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 2465 protein, preferably a mammalian 2465 protein, and can further include non-coding regulatory sequences, and introns.

Allelic variants of human 2465 include both functional and non-functional 2465 proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the human 2465 protein that maintain the ability to bind a 2465 ligand or substrate and/or modulate cell proliferation and/or migration mechanisms. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 2465 protein that do not have the ability to either bind a 2465 ligand or substrate and/or modulate cell proliferation and/or migration mechanisms. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion or deletion in critical residues or critical regions.

The methods of the present invention may further use non-human orthologues of the human 2465 protein. Orthologues of the human 2465 protein are proteins that are isolated from non-human organisms and possess the same 2465 ligand binding and/or modulation of cell proliferation and/or migration mechanisms of the human 2465 protein. Orthologues of the human 2465 protein can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2.

Moreover, nucleic acid molecules encoding other 2465 family members and, thus, which have a nucleotide sequence which differs from the 2465 sequence of SEQ ID NO:1 are intended to be within the scope of the invention. For example, another 2465 cDNA can be identified based on the nucleotide sequence of human 2465. Moreover, nucleic acid molecules encoding 2465 proteins from different species, and which, thus, have a nucleotide sequence which differs from the 2465 sequence of SEQ ID NO:1 are intended to be within the scope of the invention. For example, a mouse 2465 cDNA can be identified based on the nucleotide sequence of human 2465.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the 2465 cDNA of the invention can be isolated based on their homology to the 2465 nucleic acid disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic

variants and homologues of the 2465 cDNA of the invention can further be isolated by mapping to the same chromosome or locus as the 2465 gene.

Accordingly, in another embodiment, the methods of the invention include the use of an isolated nucleic acid molecule that is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 1000, 1200, or more nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, more preferably at 60°C, and even more preferably at 65°C. Ranges intermediate to the above-recited values, e.g., at 60-65 °C or at 55-60 °C are also intended to be encompassed by the present invention. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the 2465 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, thereby leading to changes in the amino acid sequence of the encoded 2465 protein, without altering the functional ability of the 2465 protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1. A "non-essential" amino acid residue is a residue that can

be altered from the wild-type sequence of 2465 (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the 2465 proteins of the present invention are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the 2465 proteins of the present invention and other members of the G protein-coupled receptor family are not likely to be amenable to alteration.

Accordingly, the methods of the invention may include the use of nucleic acid molecules encoding 2465 proteins that contain changes in amino acid residues that are not essential for activity. Such 2465 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a 2465 protein identical to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 2465 protein is preferably replaced with another amino acid residue from the same side chain

family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 2465 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 2465 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant 2465 protein can be assayed for the ability to (1) interact with a non-2465 protein molecule, *e.g.*, a 2465 ligand or substrate; (2) activate a 2465-dependent signal transduction pathway; or (3) modulate cell proliferation and/or migration mechanisms, or modulate the expression of cell surface adhesion molecules. In addition to the nucleic acid molecules encoding 2465 proteins described herein, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire 2465 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding 2465. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human 2465 corresponds to SEQ ID NO:1). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 2465. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding 2465 disclosed herein (*e.g.*, SEQ ID NO:1), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 2465 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 2465 mRNA. For example, the antisense oligonucleotide can be complementary to

the region surrounding the translation start site of 2465 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

In yet another embodiment, the 2465 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs"

refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* Proc. Natl. Acad. Sci. 93: 14670-675.

PNAs of 2465 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 2465 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of 2465 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of 2465 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a

stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

9. <u>Isolated 2465 Proteins and Anti-2465 Antibodies</u>

The methods of the invention include the use of isolated 2465 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-2465 antibodies.

Isolated proteins of the present invention, preferably 2465 proteins, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical.

Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently identical.

As used interchangeably herein, a "2465 activity", "biological activity of 2465" or "functional activity of 2465", refers to an activity exerted by a 2465 protein, polypeptide or nucleic acid molecule on a 2465 responsive cell (*e.g.*, an endothelial cell) or tissue, or on a 2465 protein substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a 2465 activity is a direct activity, such as an association with a 2465 target molecule. As used herein, a "target molecule" or "binding partner" is a molecule with which a 2465 protein binds or interacts in nature, such that 2465-mediated function is achieved. A 2465 target molecule can be a non-2465 molecule or a 2465 protein or polypeptide of the present invention. In an exemplary embodiment, a 2465 target molecule is a 2465 ligand. Alternatively, a 2465 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the 2465 protein with a 2465 ligand. Preferably, a 2465 activity is the ability to act as a signal transduction molecule and to modulate endothelial cell proliferation, differentiation, and/or migration. Accordingly, another embodiment of the invention features isolated 2465 proteins and polypeptides having a 2465 activity.

In one embodiment, native 2465 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, 2465 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a 2465 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the 2465 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of 2465 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of 2465 protein having less than about 30% (by dry weight) of non-2465 protein (also referred to herein as a "contaminating protein"),

more preferably less than about 20% of non-2465 protein, still more preferably less than about 10% of non-2465 protein, and most preferably less than about 5% non-2465 protein. When the 2465 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of 2465 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of 2465 protein having less than about 30% (by dry weight) of chemical precursors or non-2465 chemicals, more preferably less than about 20% chemical precursors or non-2465 chemicals, still more preferably less than about 10% chemical precursors or non-2465 chemicals, and most preferably less than about 5% chemical precursors or non-2465 chemicals.

As used herein, a "biologically active portion" of a 2465 protein includes a fragment of a 2465 protein which participates in an interaction between a 2465 molecule and a non-2465 molecule. Biologically active portions of a 2465 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the 2465 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length 2465 protein, and exhibit at least one activity of a 2465 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 2465 protein, *e.g.*, modulating cell proliferation mechanisms. A biologically active portion of a 2465 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200, or more amino acids in length. Biologically active portions of a 2465 protein can be used as targets for developing agents which modulate a 2465 mediated activity, *e.g.*, a cell proliferation mechanism. A biologically active portion of a 2465 protein comprises a protein in which regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 2465 protein.

In a preferred embodiment, the 2465 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 2465 protein is substantially identical to SEQ ID NO:2, and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the 2465 protein is a protein which comprises an amino acid sequence at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more identical to SEQ ID NO:2.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the 2465 amino acid sequence of SEQ ID NO:2 having 516 amino acid residues, at least 136, preferably at least 181, more preferably at least 227, even more preferably at least 272, and even more preferably at least 317, 362 or 408 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which

has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (Myers and Miller, *Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 2465 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3 to obtain amino acid sequences homologous to 2465 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The methods of the invention may also use 2465 chimeric or fusion proteins. As used herein, a 2465 "chimeric protein" or "fusion protein" comprises a 2465 polypeptide operatively linked to a non-2465 polypeptide. A "2465 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to 2465, whereas a "non-2465 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 2465 protein, *e.g.*, a protein which is different from the 2465 protein and which is derived from the same or a different organism. Within a 2465 fusion protein the 2465 polypeptide can correspond

to all or a portion of a 2465 protein. In a preferred embodiment, a 2465 fusion protein comprises at least one biologically active portion of a 2465 protein. In another preferred embodiment, a 2465 fusion protein comprises at least two biologically active portions of a 2465 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the 2465 polypeptide and the non-2465 polypeptide are fused in-frame to each other. The non-2465 polypeptide can be fused to the N-terminus or C-terminus of the 2465 polypeptide.

For example, in one embodiment, the fusion protein is a GST-2465 fusion protein in which the 2465 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 2465. In another embodiment, the fusion protein is a 2465 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 2465 can be increased through use of a heterologous signal sequence.

The 2465 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 2465 fusion proteins can be used to affect the bioavailability of a 2465 ligand. Use of 2465 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 2465 protein; (ii) mis-regulation of the 2465 gene; and (iii) aberrant post-translational modification of a 2465 protein. In one embodiment, a 2465 fusion protein may be used to treat a hepatic, bone, or cardiovascular disorder. In another embodiment, a 2465 fusion protein may be used to treat an endothelial cell disorder.

Moreover, the 2465-fusion proteins of the invention can be used as immunogens to produce anti-2465 antibodies in a subject, to purify 2465 ligands and in screening assays to identify molecules which inhibit the interaction of 2465 with a 2465 substrate.

Preferably, a 2465 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene

can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 2465-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 2465 protein.

The methods of the present invention may also include the use of variants of the 2465 protein which function as either 2465 agonists (mimetics) or as 2465 antagonists. Variants of the 2465 protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 2465 protein. An agonist of the 2465 protein can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 2465 protein. An antagonist of a 2465 protein can inhibit one or more of the activities of the naturally occurring form of the 2465 protein by, for example, competitively modulating a 2465-mediated activity of a 2465 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 2465 protein.

In one embodiment, variants of a 2465 protein which function as either 2465 agonists (mimetics) or as 2465 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 2465 protein for 2465 protein agonist or antagonist activity. In one embodiment, a variegated library of 2465 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of 2465 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential 2465 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of 2465 sequences therein. There are a variety of methods which can be used to produce libraries of potential 2465 variants from a

degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential 2465 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a 2465 protein coding sequence can be used to generate a variegated population of 2465 fragments for screening and subsequent selection of variants of a 2465 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a 2465 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 2465 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 2465 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 2465 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA 89*:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated 2465 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, an endothelial cell line, which ordinarily responds to a 2465 ligand in a particular 2465-dependent manner. The transfected cells are then contacted with a 2465 ligand and the effect of expression of the mutant on signaling by the 2465 receptor can be detected, *e.g.*, by monitoring the generation of an intracellular second messenger (*e.g.*, calcium, cAMP, IP_3, or diacylglycerol), the phosphorylation profile of intracellular proteins, cell proliferation and/or migration, the expression profile of cell surface adhesion molecules, or the activity of a 2465-regulated transcription factor. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the 2465 receptor, and the individual clones further characterized.

An isolated 2465 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind 2465 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 2465 protein can be used or, alternatively, the invention provides antigenic peptide fragments of 2465 for use as immunogens. The antigenic peptide of 2465 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of 2465 such that an antibody raised against the peptide forms a specific immune complex with 2465. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of 2465 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see Figure 2).

A 2465 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 2465 protein or a chemically synthesized 2465 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic 2465 preparation induces a polyclonal anti-2465 antibody response.

Accordingly, another aspect of the invention pertains to anti-2465 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as 2465. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 2465. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of 2465. A monoclonal antibody composition thus typically displays a single binding affinity for a particular 2465 protein with which it immunoreacts.

Polyclonal anti-2465 antibodies can be prepared as described above by immunizing a suitable subject with a 2465 immunogen. The anti-2465 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 2465. If desired, the antibody molecules directed against 2465 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-2465 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem* .255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an

immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 2465 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds 2465.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-2465 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 2465, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-2465 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with 2465 to thereby isolate immunoglobulin library members that bind 2465. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally,

examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.

Additionally, recombinant anti-2465 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, can also be used in the methods of the present invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-2465 antibody (*e.g.*, monoclonal antibody) can be used to isolate 2465 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-2465 antibody can facilitate the purification of natural 2465 from cells and of recombinantly produced 2465 expressed in host cells. Moreover, an anti-2465 antibody can be used to detect 2465 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the 2465 protein. Anti-2465 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: REGULATION OF 2465 EXPRESSION IN FIBROTIC LIVER CELLS

Transcriptional profiling was used to detect the presence of RNA transcript corresponding to human 2465 in several tissues. It was found that the corresponding orthologs of 2465 are expressed in a variety of tissues. The results of this screening are shown in Figures 4 and 6.

Reverse Transcriptase PCR (RT-PCR) was used to detect the presence of RNA transcript corresponding to human 2465 in RNA prepared from cells and tissues related to liver fibrosis. The highest expression of the gene was noted in dividing liver stellate cells, which are known to contribute to fibrosis. Quiescent stellate cells and other liver cells showed much lowered levels of expression, as shown in Figure 5.

In order to assess the fibrotic regulation of 2465 *in vivo*, three animal models for liver fibrosis were used. In one model, the bile duct of rats was surgically ligated, thus causing a fibrosis-like state in the liver by ceasing the flow of bile. RT-PCR was used to assess the expression of the rat ortholog of 2465 at several time points after bile-duct ligation. The results of this analysis are shown in Figure 7.

In another whole animal model, porcine serum was injected into rats, thus, inducing a fibrotic liver condition. RT-PCR was used to assess the expression of the rat ortholog of 2465 in the fibrotic liver. The results of this analysis are shown in Figure 8.

In a third whole animal model, a fibrotic liver condition was induced by injection of a toxin (carbon tetrachloride) into rats. RT-PCR was again used to assess the expression of the rat ortholog of 2465 in the fibrotic liver. The results of this analysis are shown in Figure 9.

EXAMPL[illegible] REGULATION OF 2465 EX[illegible]SSION IN CELLS INVOLVED IN OSTEOGENESIS

The expression of 2465 was assessed in several cell types including cells of adipocyte lineage and those of osteoblast lineage using RT-PCR. The results are shown in Figure 10.

Relative expression levels of 2465 were assessed in osteogenic cells and adipogenic cells using TaqMan PCR. The results of this analysis are shown in Figure 11.

TaqMan PCR was also used to assess the expression of 2465 in several cellular models of osteoporosis. The results of this comparison are shown in Figure 12.

Expression of 2465 was assessed in several tissues. A relatively high expression of the transcript was found in differentiated osteoblasts, and primary cultured osteoblasts. The results of this survey are shown in Figure 13.

EXAMPLE 3: EXPRESSION OF RECOMBINANT 2465 PROTEIN IN BACTERIAL CELLS

In this example, 2465 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 2465 is fused to GST and this fusion polypeptide is expressed in *E. coli, e.g.*, strain PEB199. Expression of the GST-2465 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

EXPRESSION OF RECOMBINANT 2465 PROTEIN IN COS CELLS

To express the 2465 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of

replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 2465 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 2465 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 2465 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 2465 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 2465 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 2465-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the VR-3 or VR-5 polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S - cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents

(RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 2465 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 2465 polypeptide is detected by radiolabelling and immunoprecipitation using a 2465 specific monoclonal antibody.

EXAMPLE 5: EXPRESSION OF 2465 IN ISOLATED HUMAN VESSELS

Reverse Transcriptase PCR (RT-PCR) was used to detect the presence of RNA transcripts corresponding to human 2465 in mRNA prepared from isolated human vessels. The highest expression of the gene was noted in endothelial cells and smooth muscle cells, consistent with a role of this molecule in vascular functions, while expression in adipose tissue (which may contaminate vessel preparations) was low (see Figure 14).

EXAMPLE 6: REGULATION OF 2465 EXPRESSION IN ISOLATED HUMAN VESSELS

Human umbilical vein endothelial cells (HUVEC's) were cultured *in vitro* under standard conditions, described in, for example, U.S. Patent 5,882,925. Experimental cultures were then exposed to laminar shear stress (LSS) conditions by culturing the cells in a specialized apparatus containing liquid culture medium. Static cultures grown in the same medium served as controls. The *in vitro* LSS treatment at 10 dyns/cm^2 was performed for 24 hours and was designed to simulate the shear stress generated by blood flow in a straight, healthy artery.

The effect of LSS on 2465 expression in endothelial cells was assessed by Taqman analysis. 2465 gene expression was significantly induced in HUVECs exposed to LSS. (Figure 15, bars 1-4).

In another study, HUVEC or microvascular endothelial cells cultured from human heart (HMVEC-C) or lung (HMVEC-L) were harvested while rapidly proliferating ("prolif"), or after they had reached confluence in their regular growth medium ("conf") or in growth factor depleted medium ("-GF") (set forth in Figure 15). Bars which are grouped together refer to a particular cell preparation. Upregulation of 2465 was observed under proliferating conditions (compare bar 5 to bars 6 and 7, bar 9 to bar 10, bar 11 to bar 12, and bar 13 to bars 14 and 15).

In addition, HUVEC cultures were treated with human IL-1β, a factor known to be involved in the inflammatory response, in order to mimic the physiologic conditions involved in the atherosclerotic state. Stimulation of endothelial cells with IL-1β induces the expression of several inflammatory markers. 2465 expression was upregulated by treatment with IL-1β (compare bar 8 to bar 6, which is the untreated control).

Collectively, these data indicate that 2465 may be involved in the regulation of endothelial cell processes such as proliferation, which are relevant to angiogenesis and the development of atherosclerosis. The data also indicate that 2465 may play a role in vascular functions such as in the control of vascular tone.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1.

2. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.

3. An isolated nucleic acid molecule comprising the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number ______.

4. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.

5. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1, or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 30 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

6. An isolated nucleic acid molecule which hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions.

7. An isolated nucleic acid molecule comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

8. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5, and a nucleotide sequence encoding a heterologous polypeptide.

9. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

10. The vector of claim 9, which is an expression vector.

11. A host cell transfected with the expression vector of claim 10.

12. A method of producing a polypeptide comprising culturing the host cell of claim 11 in an appropriate culture medium to, thereby, produce the polypeptide.

13. An isolated polypeptide selected from the group consisting of:

a)a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acids of SEQ ID NO:2;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 under stringent conditions;

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1; and

d) a polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:2.

14. The isolated polypeptide of claim 13 comprising the amino acid sequence of SEQ ID NO:2.

15. The polypeptide of claim 13, further comprising heterologous amino acid sequences.

16. An antibody which selectively binds to a polypeptide of claim 13.

17. A method for detecting the presence of a polypeptide of claim 13 in a sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 13 in the sample.

18. The method of claim 17, wherein the compound which binds to the polypeptide is an antibody.

19. A kit comprising a compound which selectively binds to a polypeptide of claim 13 and instructions for use.

20. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to a complement of the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to the complement of the nucleic acid molecule in the sample to thereby detect the presence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in the sample.

21. The method of claim 20, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

22. A kit comprising a compound which selectively hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 and instructions for use.

23. A method for identifying a compound which binds to a polypeptide of claim 13 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

24. The method of claim 23, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for 2465 activity.

25. A method for modulating the activity of a polypeptide of claim 13 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

26. A method for identifying a compound which modulates the activity of a polypeptide of claim 13 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

27. The method of claim 26, wherein said activity is modulation of a hepatic, bone, or cardiovascular function.

28. A method for identifying a compound which modulates a hepatic, bone, or cardiovascular function comprising:

a) contacting the polypeptide of claim 13, or a cell expressing the polypeptide with a test compound; and

b) identifying the compound as a modulator of a hepatic, bone, or cardiovascular function by determining the effect of the test compound on the activity of the polypeptide.

29. A method for identifying a compound which modulates liver fibrosis, osteoporosis, or atherosclerosis comprising:

a) contacting the polypeptide of claim 13, or a cell expressing the polypeptide with a test compound; and

b) identifying the compound as a modulator of liver fibrosis, osteoporosis, or atherosclerosis by determining the effect of the test compound on the activity of the polypeptide.

30. A method for treating a subject having a hepatic disorder, a bone disorder, or a cardiovascular disorder comprising administering to the subject a 2465 modulator, thereby treating said subject having a hepatic disorder, a bone disorder, or a cardiovascular disorder.

31. A method for treating a subject having a hepatic disorder, a bone disorder, or a cardiovascular disorder comprising administering to the subject a 2465 modulator, wherein the 2465 modulator is the modulator identified by the method of claim 26, thereby treating said subject having a hepatic disorder, a bone disorder, or a cardiovascular disorder.

32. The method of claim 30, wherein the 2465 modulator is a small molecule.

33. The method of claim 30, wherein said 2465 modulator is administered in a pharmaceutically acceptable formulation.

34. The method of claim 30, wherein said 2465 modulator is administered using a gene therapy vector.

35. The method of 30, wherein the 2465 modulator is capable of modulating 2465 polypeptide activity.

TGGGGGCGTCCTCCTTCGTC[illegible]GCCCGGCTGTCAAGCTGTGTTCTAGCGGCCGAG[illegible]CGAGGGGGGCTAAGAAAGG
GGGCGCCCAGCCATGCAGAGGCAAAAAGGCGCTGCGGAACGGGGTCCCCGTCGCCAGTGCTGAGGCAGGAGGTCGGAGC
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CCGCTAAGGAGAGGGGTCGAGTGCGTCAGCCCGAGGGGACTGGAGAGGGATGCCCTAGCCCTCGAGGGGCGGAGGACCC
GCGGTTGAAGGAGGCAGCGGGAGCGGAGAGCGCCCTCCTTGACCATCGAATGCCTCCTTCTGTGTTTCCATTCCTGTCG
AGTGGGCTGGGCCACGCTGACCACCCTGGAGGAGGGACGGACGACGCTCGGCGGGCTCTGACCGTGCCGCCTTCTTGTG
GCTGCTGACTGGGATCCAGGAGGGAGTGGGCATGGGGCGCAGCCGCGCCTCCCTCCCTCCCCGCCTCCCGGGCGCCGGG
GTTGGCGATGTGGAGACGTGAGGGGACCCGTCGGCTGCTCCGGCTTCTCCAGGACTCCGCCAGGCGCCCGCGCGTCCCT
CCTCACCCGGAGGAGGAGAGGCTCCGCGGGGCTCCGAGGCGGCGGCGCGCGGAGCCGGAGTCCCAGCCTCGCC

M G H N G S W I S P N A S E P H N A S G 20
ATG GGA CAT AAC GGG AGC TGG ATC TCT CCA AAT GCC AGC GAG CCG CAC AAC GCG TCC GGC 60

A E A A G V N R S A L G E F G E A Q L Y 40
GCC GAG GCT GCG GGT GTG AAC CGC AGC GCG CTC GGG GAG TTC GGC GAG GCG CAG CTG TAC 120

R Q F T T T V Q V V I F I G S L L G N F 60
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M V L W S T C R T T V F K S V T N R F I 80
ATG GTG TTA TGG TCA ACT TGC CGC ACA ACC GTG TTC AAA TCT GTC ACC AAC AGG TTC ATT 240

K N L A C S G I C A S L V C V P F D I I 100
AAA AAC CTG GCC TGC TCG GGG ATT TGT GCC AGC CTG GTC TGT GTG CCC TTC GAC ATC ATC 300

L S T S P H C C W W I Y T M L F C K V V 120
CTC AGC ACC AGT CCT CAC TGT TGC TGG TGG ATC TAC ACC ATG CTC TTC TGC AAG GTC GTC 360

K F L H K V F C S V T I L S F P A I A L 140
AAA TTT TTG CAC AAA GTA TTC TGC TCT GTG ACC ATC CTC AGC TTC CCT GCT ATT GCT TTG 420

D R Y Y S V L Y P L E R K I S D A K S R 160
GAC AGG TAC TAC TCA GTC CTC TAT CCA CTG GAG AGG AAA ATA TCT GAT GCC AAG TCC CGT 480

E L V M Y I W A H A V V A S V P V F A V 180
GAA CTG GTG ATG TAC ATC TGG GCC CAT GCA GTG GTG GCC AGT GTC CCT GTG TTT GCA GTA 540

T N V A D I Y A T S T C T E V W S N S L 200
ACC AAT GTG GCT GAC ATC TAT GCC ACG TCC ACC TGC ACG GAA GTC TGG AGC AAC TCC TTG 600

G H L V Y V L V Y N I T T V I V P V V V 220
GGC CAC CTG GTG TAC GTT CTG GTG TAT AAC ATC ACC ACG GTC ATT GTG CCT GTG GTG GTG 660

V F L F L I L I R R A L S A S Q K K K V 240
GTG TTC CTC TTC TTG ATA CTG ATC CGA CGG GCC CTG AGT GCC AGC CAG AAG AAG AAG GTC 720

Fig. 1

SUBSTITUTE SHEET (RULE 26)

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ATC ATA GCA GCG CTC CGG ACC CCA CAG AAC ACC ATC TCT ATT CCC TAT GCC TCC CAG CGG  780

 E   A   E   L   H   A   T   L   L   S   M   V   M   V   F   I   L   C   S   V   280
GAG GCC GAG CTG CAC GCC ACC CTG CTC TCC ATG GTG ATG GTC TTC ATC TTG TGT AGC GTG  840

 P   Y   A   T   L   V   V   Y   Q   T   V   L   N   V   P   D   T   S   V   F   300
CCC TAT GCC ACC CTG GTC GTC TAC CAG ACT GTG CTC AAT GTC CCT GAC ACT TCC GTC TTC  900

 L   L   L   T   A   V   W   L   P   K   V   S   L   L   A   N   P   V   L   F   320
TTG CTG CTC ACT GCT GTT TGG CTG CCC AAA GTC TCC CTG CTG GCA AAC CCT GTT CTC TTT  960

 L   T   V   N   K   S   V   R   K   C   L   I   G   T   L   V   Q   L   H   H   340
CTT ACT GTG AAC AAA TCT GTC CGC AAG TGC TTG ATA GGG ACC CTG GTG CAA CTA CAC CAC 1020

 R   Y   S   R   R   N   V   V   S   T   G   S   G   M   A   E   A   S   L   E   360
CGG TAC AGT CGC CGT AAT GTG GTC AGT ACA GGG AGT GGC ATG GCT GAG GCC AGC CTG GAA 1080

 P   S   I   R   S   G   S   Q   L   L   E   M   F   H   I   G   Q   Q   Q   I   380
CCC AGC ATA CGC TCG GGT AGC CAG CTC CTG GAG ATG TTC CAC ATT GGG CAG CAG CAG ATC 1140

 F   K   P   T   E   D   E   E   E   S   E   A   K   Y   I   G   S   A   D   F   400
TTT AAG CCC ACA GAG GAT GAG GAA GAG AGT GAG GCC AAG TAC ATT GGC TCA GCT GAC TTC 1200

 Q   A   K   E   I   F   S   T   C   L   E   G   E   Q   G   P   Q   F   A   P   420
CAG GCC AAG GAG ATA TTT AGC ACC TGC CTG GAG GGA GAG CAG GGG CCA CAG TTT GCG CCC 1260

 S   A   P   P   L   S   T   V   D   S   V   S   Q   V   A   P   A   A   P   V   440
TCT GCC CCA CCC CTG AGC ACA GTG GAC TCT GTA TCC CAG GTG GCA CCG GCA GCC CCT GTG 1320

 E   P   E   T   F   P   D   K   Y   S   L   Q   F   G   F   G   P   F   E   L   460
GAA CCT GAA ACA TTC CCT GAT AAG TAT TCC CTG CAG TTT GGC TTT GGG CCT TTT GAG TTG 1380

 P   P   Q   W   L   S   E   T   R   N   S   K   K   R   L   L   P   P   L   G   480
CCT CCT CAG TGG CTC TCA GAG ACC CGA AAC AGC AAG AAG CGG CTG CTT CCC CCC TTG GGC 1440

 N   T   P   E   E   L   I   Q   T   K   V   P   K   V   G   R   V   E   R   K   500
AAC ACC CCA GAA GAG CTG ATC CAG ACA AAG GTG CCC AAG GTA GGC AGG GTG GAG CGG AAG 1500

 M   S   R   N   N   K   V   S   I   F   P   K   V   D   S   *                   516
ATG AGC AGA AAC AAT AAA GTG AGC ATT TTT CCA AAG GTG GAT TCC TAG                 1548

CAAGGATTGTAAATTCTTGGAAGCAACGGGGGGCTTCCATATTCCCACCAGAGTGTGGGAATGCTGTGGCCATGTGATT
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TAAAATCCAGTGTCCTCTTTATTGAGGGAGTATATGTATCCATCTCAGTGATCCATGTCCTTAGTGAAGTCCACATTAT
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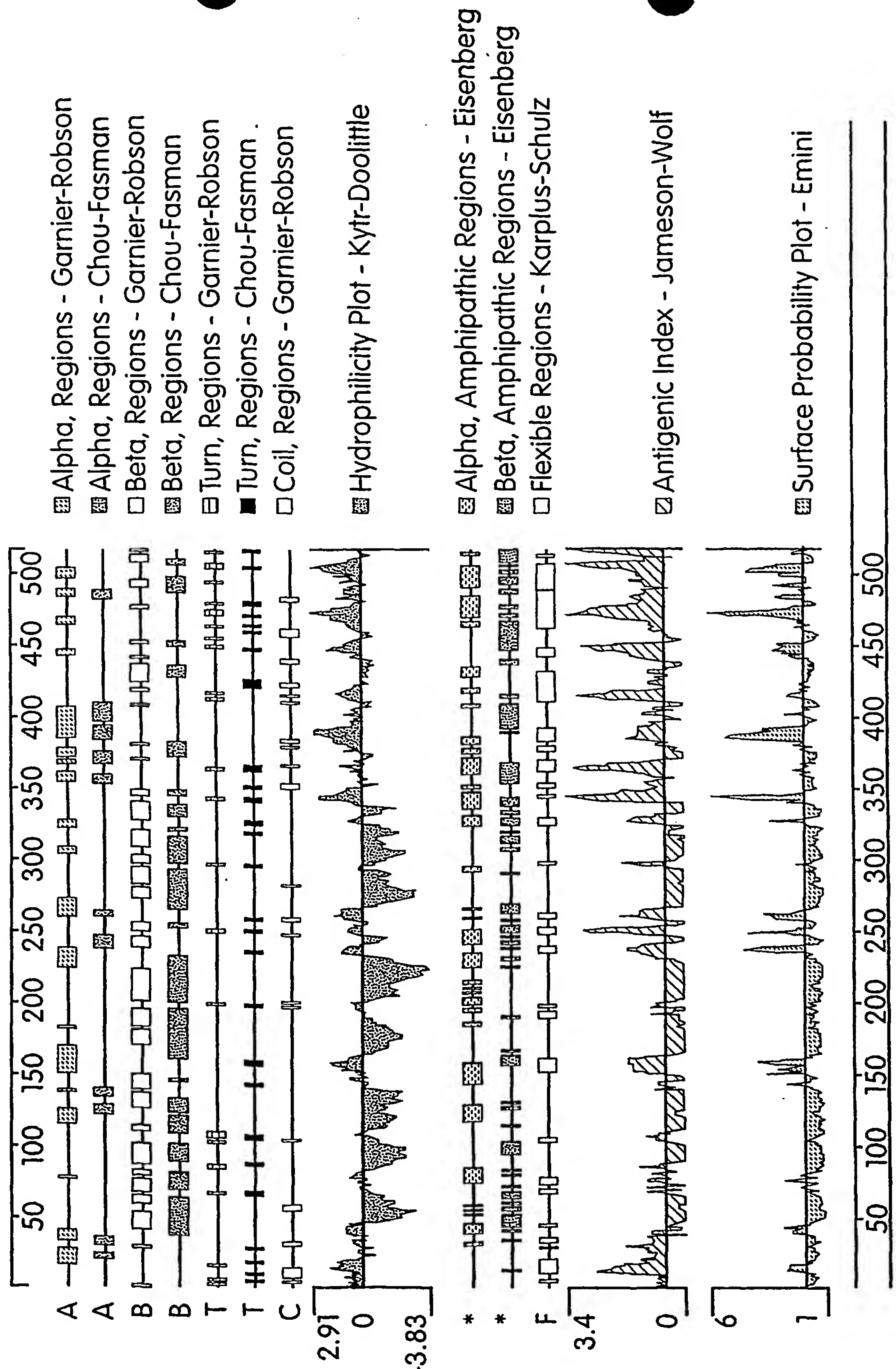
GGAATTC
```

Fig 1A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Alpha, Regions - Chou-Fasman
Beta, Regions - Garnier-Robson
Beta, Regions - Chou-Fasman
Turn, Regions - Garnier-Robson
Turn, Regions - Chou-Fasman
Coil, Regions - Garnier-Robson
Hydrophilicity Plot - Kytr-Doolittle
Alpha, Amphipathic Regions - Eisenberg
Beta, Amphipathic Regions - Eisenberg
Flexible Regions - Karplus-Schulz
Antigenic Index - Jameson-Wolf
Surface Probability Plot - Emini
A
A
B
B
T
T
C
2.91
0
-3.83
*
*
F
3.4
0
6
1
50
100
150
200
250
300
350
400
450
500

Fig. 2

```
D38449-GPCRhuman   MGHNGSWISPNASEPHNASGAEAAGVNRSALGEFGEAQLYRQFTTTVQVVIFIGSLLGNF
2465               MGHNGSWISPNASEPHNASGAEAAGVNRSALGEFGEAQLYRQFTTTVQVVIFIGSLLGNF
D38450_GPCRrat     ------------------------------------------------------------

D38449-GPCRhuman   MVLWSTCRTTVFKSVTNRFIKNLACSGICASLVCVPFDIILSTSPHCCWWIYTMLFCKVV
2465               MVLWSTCRTTVFKSVTNRFIKNLACSGICASLVCVPFDIILSTSPHCCWWIYIMLFCKVV
D38450_GPCRrat     -------RTTVFKSVTNRFIKNLACSGICASVVCVPFDIILSTSPHCCWWIYTMLFCKVL
                          ************************:***************************:

D38449_GPCRhuman   KFLHKVFCSVTILSFPAIALDRYYSVLYPLERKISDAKSRELVMYIWAHAVVASVPVFAV
2465               KFLHKVFCSVTILSFPAIALDRYYSVLYPLERKISDAKSRELVMYIWAHAVVASVPVFAV
D38450_GPCRrat     KFLHKVFCSVTVLSFPAIALDRYYSVLYPLERKISDAKSRELVMYIWAHAVVASVPVFAV
                   ***********:************************************************

D38449_GPCRhuman   TNVADIYATSTCTEVWSNSLGHLVYVLVYNITTVIVPVVVVFLFLILIRRALSASQKKKV
2465               TNVADIYATSTCTEVWSNSLGHLVYVLVYNITTVIVPVVVVFLFLILIRRALSASQKKKV
D38450_GPCRrat     TNVADIYATSTCTEVWSNSLGHLVYVLIYNVTTVIVPAVVFLFLILIRRALSASQKKKV
                   ***************************:**:******.**********************

D38449_GPCRhuman   IIAALRTPQNTISIPYASQREAELHATLLSMVMVFILCSVPYATLVVYQTVLNVPDTSVF
2465               IIAALRTPQNTISIPYASQREAELHATLLSMVMVFILCSVPYATLVVYQTVLNVPDTSVF
D38450_GPCRrat     IIAALRTPQNTISIPYASQREAELHATLLSMVTVFILCSVPYATLVVYQTVLNVPNTSVF
                   ******************************** **********************:****

D38449-GPCRhuman   LLLTAVWLPKVSLLANPVLFLTVNKSVRKCLIGTLVQLHHRYSRRNVVSTGSGMAEASLE
2465               LLLTAVWLPKVSLLANPVLFLTVNKSVRKCLIGTLVQLHHRYSRRNVVSTGSGMAEASLE
D38450_GPCRrat     LLLTAIWLPKVSLLANPVLFLTVNRSVRKCLVGTLVQLHHRYSRRNVVSTGSGVVEPSLE
                   *****:******************:******:*********************:.*.***

D38449_GPCRhuman   PSIRSGSQLLEMFHIGQQQIFKPTEDEEESEAKYIGSADFQAKEIFSTCLEGEQGPQFAP
2465               PSIRSGSQLLEMFHIGQQQIFKPTEDEEESEAKYIGSADFQAKEIFSTCLEGEQGPQFAP
D38450_GPCRrat     PSMRSGSQLLEMFHIGQQQIFKPSEDEEESEAKYLGSTDFQAKEVLTSSPEGEES-QLAP
                   **:********************:**********:**:******:::.  ***:. *:**

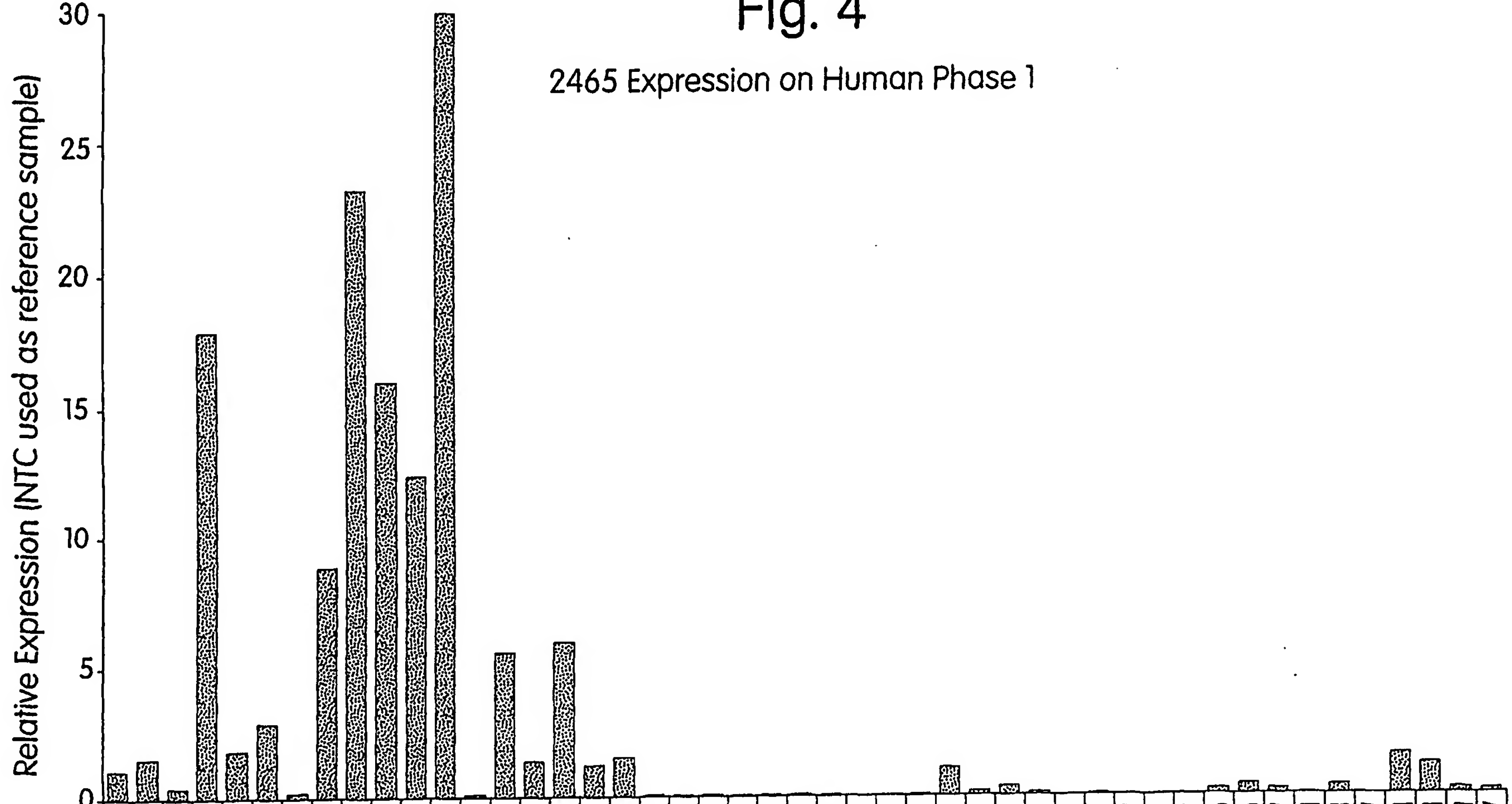
D38449-GPCRhunan   SAPPLSTVDSVSQVAPAAPVEPETFPDKYSLQFGFGPFELPPQWLSETRNSKKRLLPPLG
2465               SAPPLSTVDSVSQVAPAAPVEPETFPDKYSLQFGFGPFELPPQWLSETRNSKKRLLPPLG
D38450_GPCRrat     SVPPPGTVDSVSRVSPVAPVEPGIFPDKYSLQFGFGPFELPPQWLSETRNSKKRLLPPLG
                   *.** .******:*:*.*****  ************************************

D38449_GPCRhuman   NTPEELIQTKVPKVGRVERKMSRNNKVSIFPKVDS
2465               NTPEELIQTKVPKVGRVERKMSRNNKVSIFPKVDS
D38450_GPCRrat     NTPEELIQTKVPRVSRVERKMSRNNKVSIFPKVDS
                   ************:*.********************
```

Fig. 3

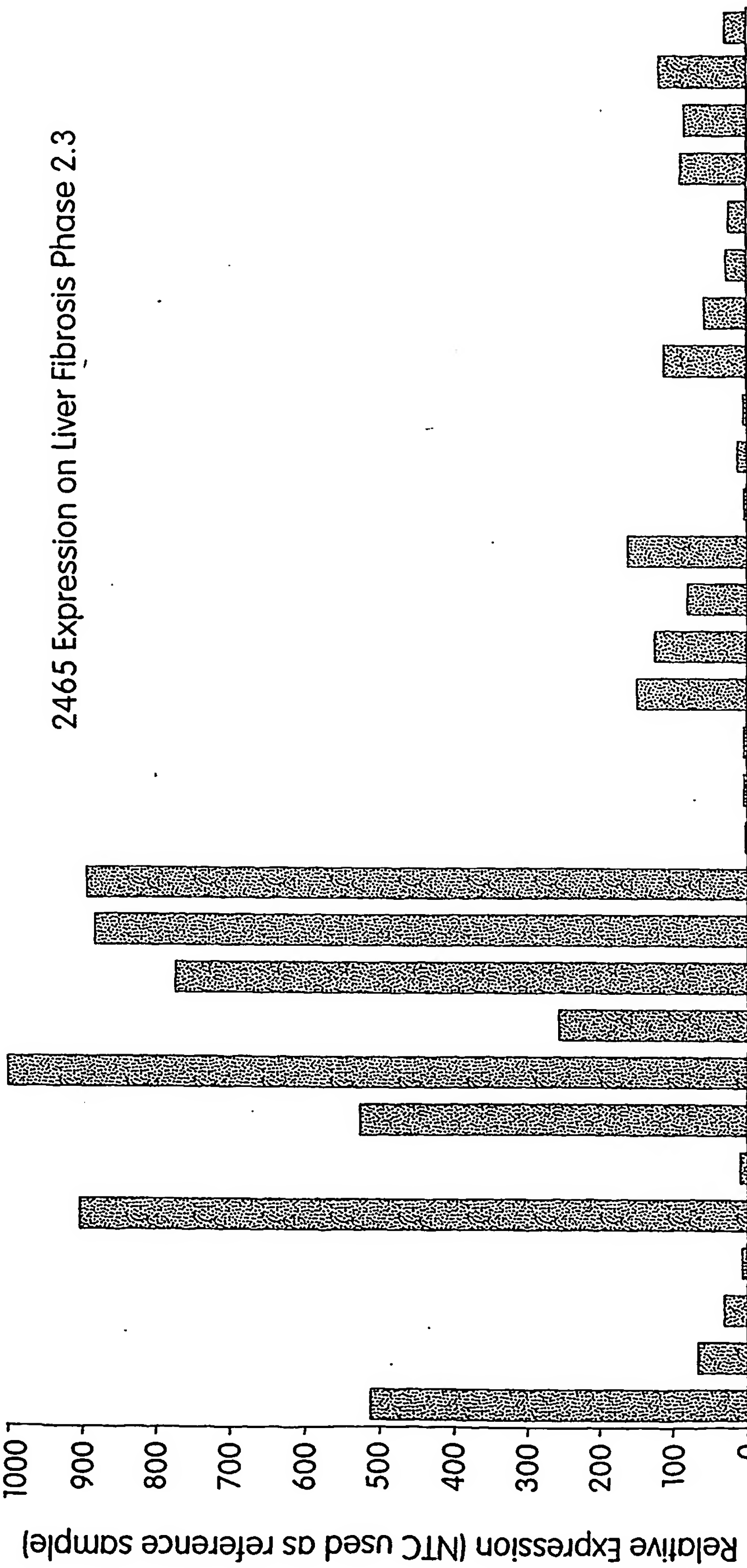
Fig. 4

2465 Expression on Human Phase 1



Sample	2465	Beta2	Rel EXP
Heart	28	19	1
Lung	27	17	2
Liver Pool	29	18	0
Brain	28	23	18
Fetal Liver	30	20	2
Spleen	24	16	3
Grans.	34	21	0
NHDF mock	26	19	9
NHLF mock	23	18	23
NHLF TGF	24	18	16
Hep4	23	17	12
Pass Stell	23	18	33
Control liver20 0	31	18	0
LF/ND R 191	27	20	6
LF/ND R 193	28	19	1
LF/ND R 079	26	19	6
Tonsil	26	17	1
LN NDR 173	28	19	2
TH1 MP39	31	16	0
TH2 MP39	30	16	0
TH1 MP21	30	16	0
TH2 MP21	30	16	0
CD4	32	17	0
CD8	33	19	0
CD19	30	16	0
CD3 MP42	37	16	0
MBM	34	17	0
CD34+MPB	32	19	0
ABM CD34+	31	21	1
Cord Blood	35	23	0
Erythroid	30	19	0
Meg	32	18	0
Neut d14	37	17	0
CD14-LF32	32	17	0
MBM CD11b	34	16	0
CD14 #7	35	16	0
PBMC	32	15	0
G2	33	21	0
G2.21 5	31	20	0
MA101	30	18	0
HL60	37	19	0
K562	32	20	0
Molt 4	40	19	0
Hep3B Normoxia	29	19	2
Hep3B Hypoxia	28	18	1
BM GPA+	34	21	0
ABM 34+	31	18	0

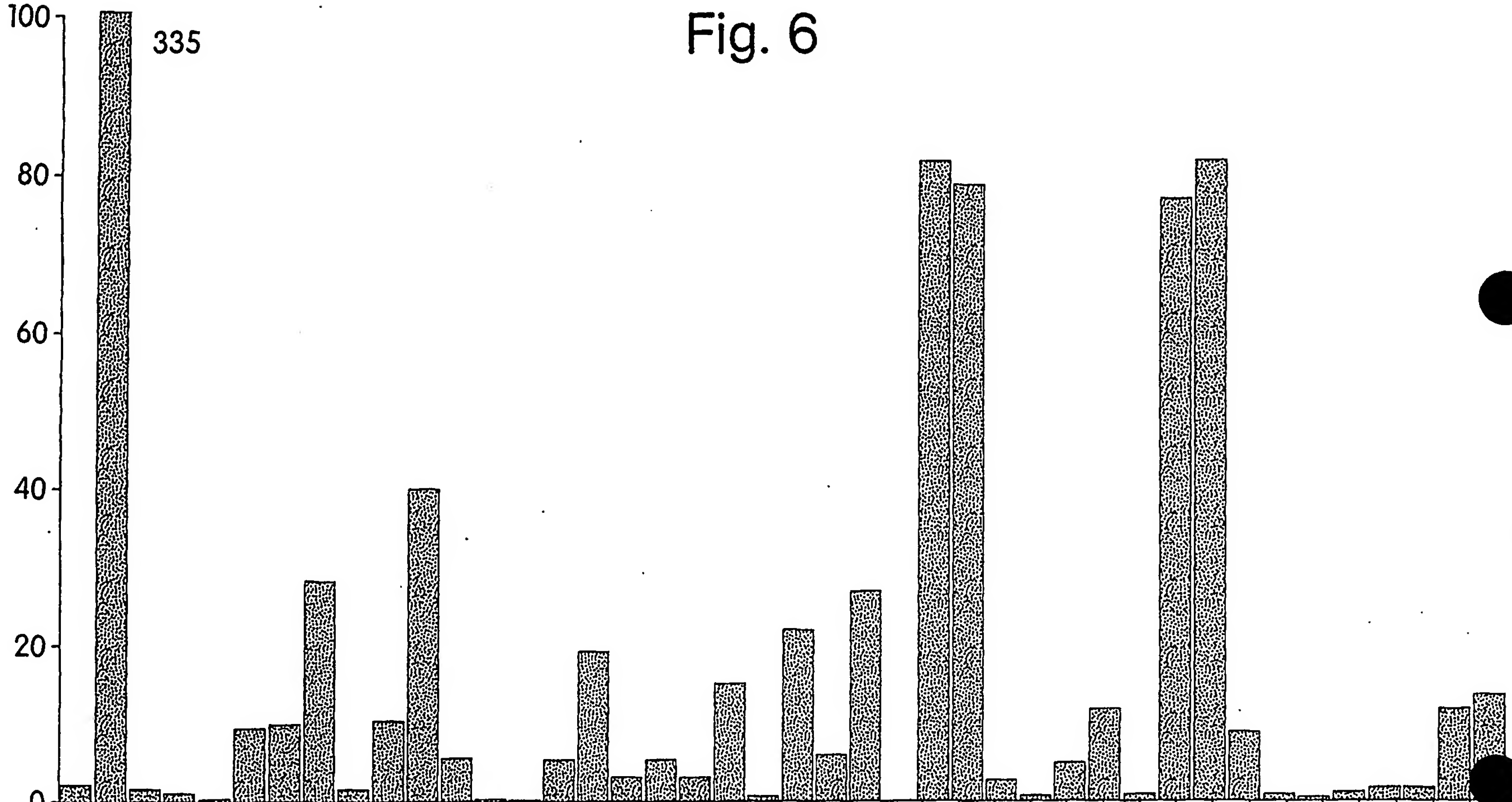
2465 Expression on Liver Fibrosis Phase 2.3



Sample	2465	Beta2	Rel EXP
Brain	29	22	511
Heart	28	18	71
Lung	28	17	34
Liver Pool	31	18	9
Passage d Stellate	24	18	908
Quiescent Stellate	33	21	14
Stellate	26	19	529
Stellate/FSB	25	20	1338
NHDF Mock 48 hrs.	26	18	262
NHDF TGF 48 hrs.	25	19	779
NHLF Mock 48 hrs.	24	17	889
NHLF TGF 48 hrs.	23	17	901
HepG2 Mock 48 hrs.	33	19	3
HepG2 TGF 48 hrs.	34	20	5
NC Heps	32	18	6
Hep-1 Control	26	17	155
Hep-2 PMA/Iono 6hr.	26	17	129
Hep-3 TGF 24 hrs.	27	18	87
Hep-4 TGF 48 hrs.	26	17	165
Liver NDR 200 #2	33	19	4
Liver CHT 339 #4	31	19	18
Liver Pit 260 #6	31	17	4
LF/NDR 079	27	18	115
LF/NDR 141	32	22	62
LF/NDR 156	30	19	31
LF/NDR 190	32	21	26
LF/NDR 191	28	19	92
LF/NDR 192	30	21	87
LF/NDR 194	33	25	120
LF/NDR 225	30	19	29

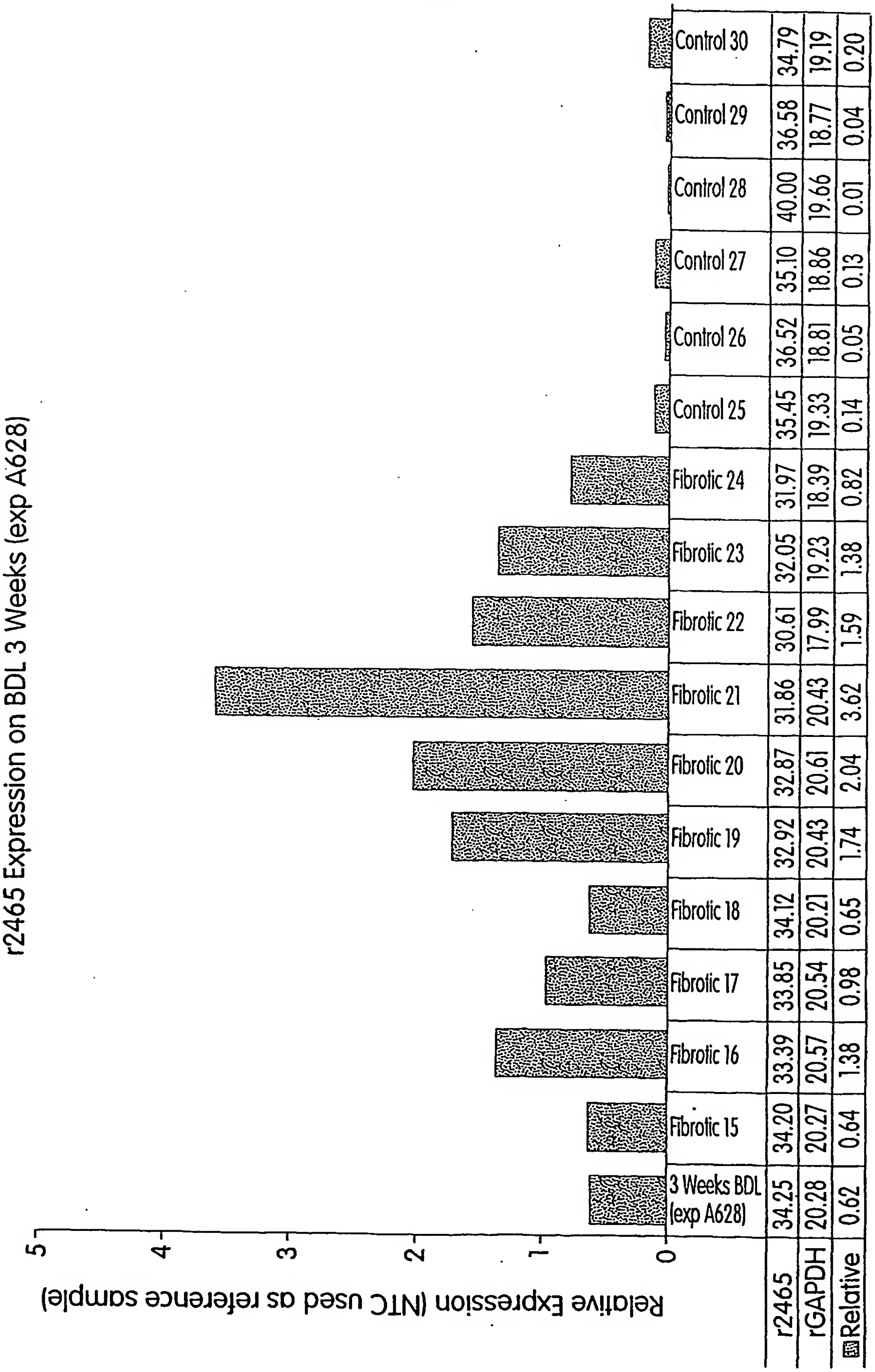
Fig. 5

Fig. 6



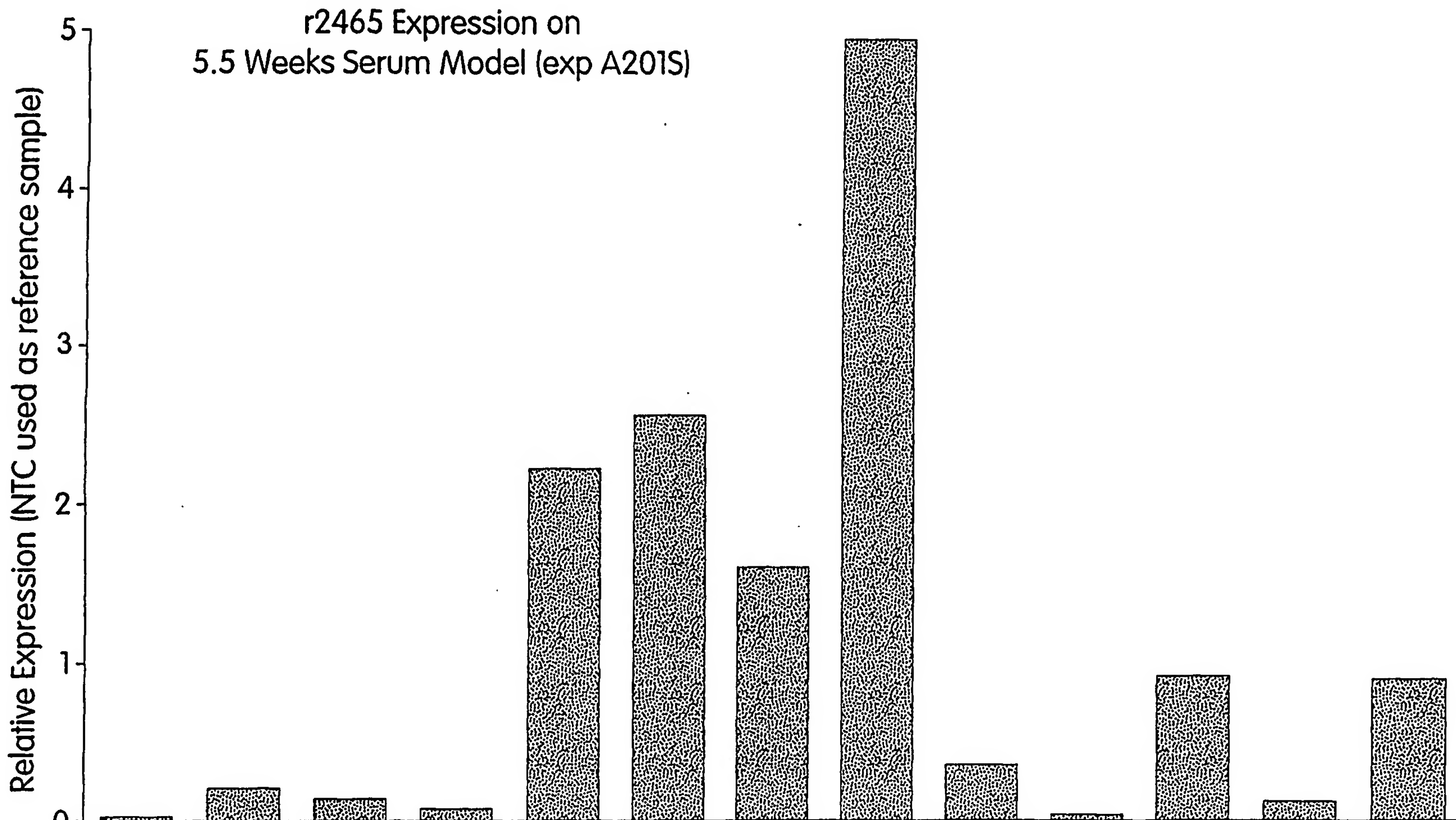
	Prostate MPI	Osteoclasts	Liver MPI 154	Liver MPI 75	Breast	Sk. Muscle	Sk. Muscle	Brain MPI 515	Colon MPI 176	Colon MPI 383/4	Heart MPI 53	Ovary MPI 423	Ovary MPI 415	Kidney MPI	Kidney MPI	Lung MPI 28	Lung MPI 131	Vein MPI 134	Vein MPI 135	Adipose MPI
2465 Average	29.8	25.4	32.7	31.3	35.4	28.0	24.4	26.5	36.0	36.4	30.2	32.5	33.7	27.1	26.7	25.3	27.6	28.5	28.9	29.7
B2 Average	20.8	23.8	23.3	21.3	23.2	21.3	17.7	21.4	26.7	29.8	22.7	20.5	20.7	19.5	21.0	17.0	20.0	20.2	22.8	19.4
Delta	9.0	1.6	9.4	10.0	12.2	6.8	6.7	5.2	9.3	6.6	7.5	12.0	13.0	7.6	5.7	8.3	7.5	8.3	6.0	10.4
REL	1.9	335.6	1.5	1.0	0.2	9.2	9.8	28.2	1.6	10.3	5.5	0.2	0.1	5.3	19.3	3.2	5.4	3.3	15.4	0.8

	Adipose MPI	Sm Intestine	Thyroid MPI	Bone Marrow	Skin MPI 572	Testis MPI 33/78	Placenta MPI	Fetal Liver MPI	Fetal Liver MPI	Fetal Heart MPI	Undif Osteoblasts	Dif Osteoblasts	Prim Cult Osteo	Spinal cord MPI	Cervix MPI	Spleen MPI	Spinal Cord MPI	Thymus MPI	Tonsil MPI 396	Lymph Node
2465 Average	27.2	29.9	27.5	34.9	24.2	24.1	31.8	29.2	29.7	25.5	22.1	22.7	25.3	29.7	31.3	28.2	27.5	28.9	27.4	24.1
B2 Average	21.7	22.5	22.3	20.1	20.6	20.5	23.3	18.8	22.1	19.1	18.4	19.1	18.5	19.4	19.5	18.2	18.2	19.4	20.9	17.9
Delta	5.5	7.4	5.2	14.8	3.6	3.7	8.5	10.4	7.7	6.4	3.7	3.6	6.8	10.3	11.8	9.9	9.3	9.6	6.5	6.2
REL	22.3	6.0	27.0	0.0	81.9	78.8	2.7	0.7	5.0	11.9	76.9	81.9	8.7	0.8	0.3	1.0	1.5	1.3	11.4	13.2



	3 Weeks BDL (exp A628)	Fibrotic 15	Fibrotic 16	Fibrotic 17	Fibrotic 18	Fibrotic 19	Fibrotic 20	Fibrotic 21	Fibrotic 22	Fibrotic 23	Fibrotic 24	Control 25	Control 26	Control 27	Control 28	Control 29	Control 30
r2465	34.25	34.20	33.39	33.85	34.12	32.92	32.87	31.86	30.61	32.05	31.97	35.45	36.52	35.10	40.00	36.58	34.79
rGAPDH	20.28	20.27	20.57	20.54	20.21	20.43	20.61	20.43	17.99	19.23	18.39	19.33	18.81	18.86	19.66	18.77	19.19
Relative	0.62	0.64	1.38	0.98	0.65	1.74	2.04	3.62	1.59	1.38	0.82	0.14	0.05	0.13	0.01	0.04	0.20

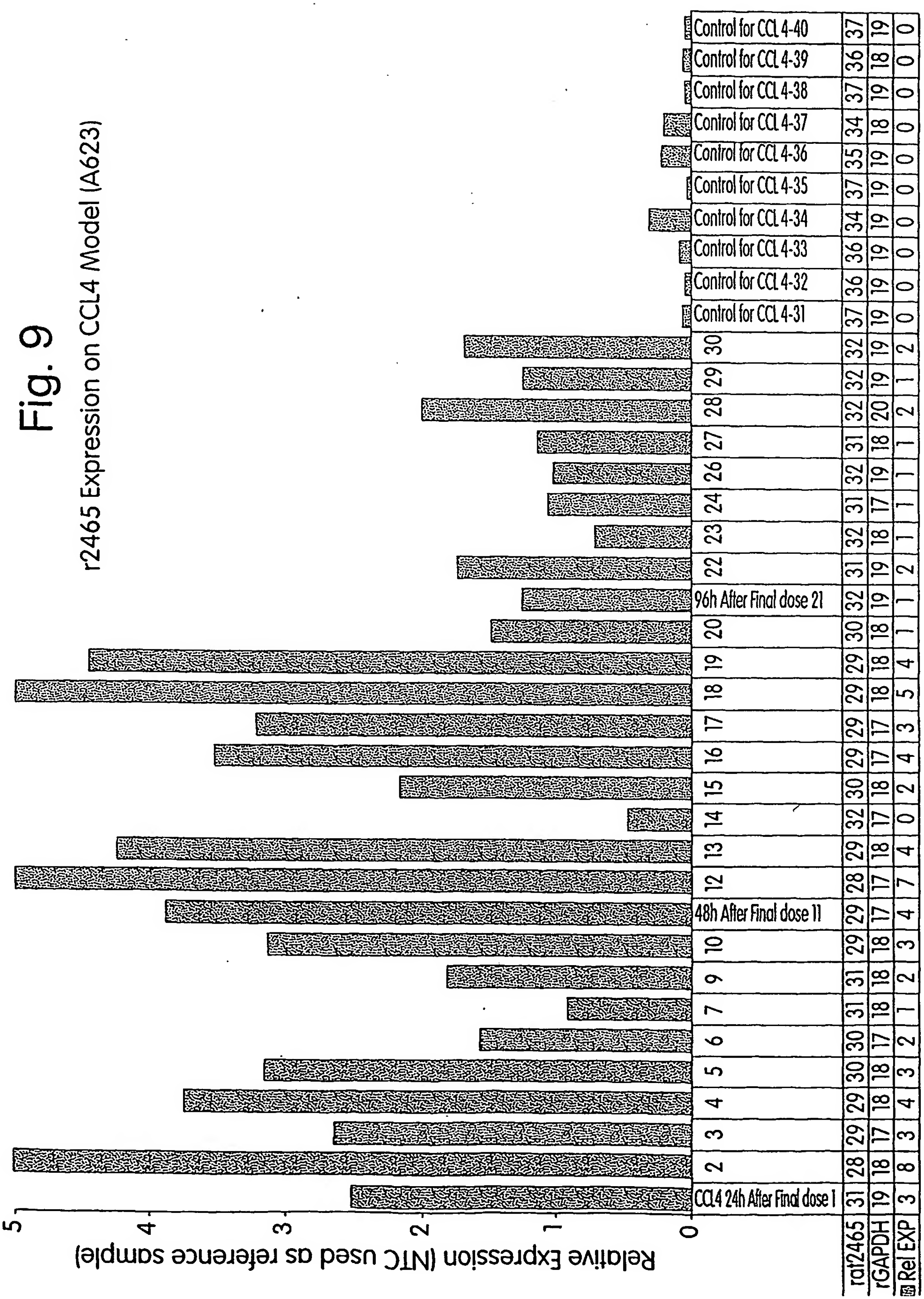
Fig. 7



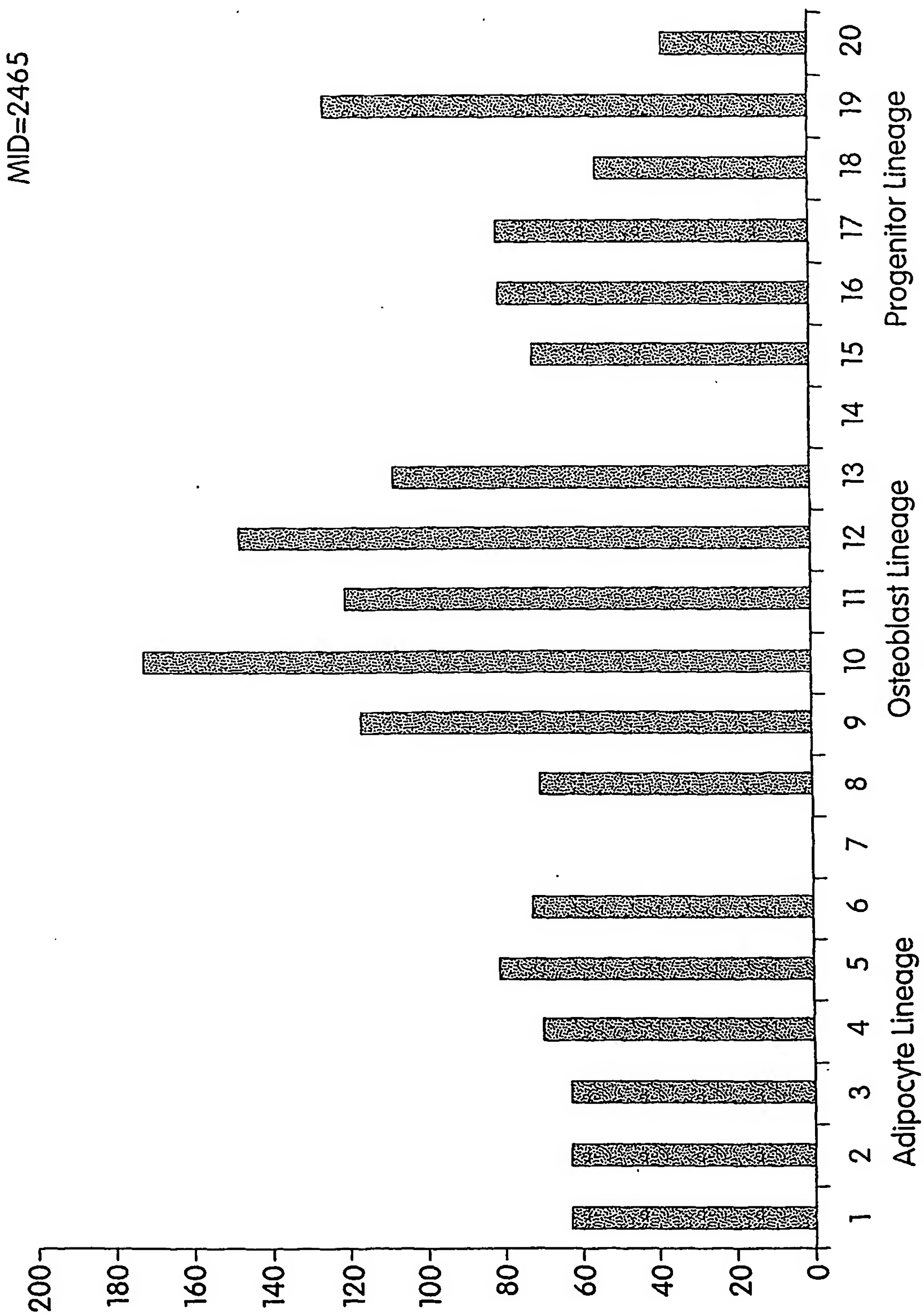
	5.5 Serum (exp A201S Control 1	5.5 Serun Control 3	5.5 Serun Control 4	5.5 Serun Control 5	5.5 Serun Fibrotic 7	5.5 Serun Fibrotic 8	5.5 Serun Fibrotic 9	5.5 Serun Fibrotic 10	5.5 Serun Fibrotic 16	5.5 Serun Fibrotic 17	5.5 Serun Fibrotic 18	5.5 Serun Fibrotic 19	5.5 Serun Fibrotic 20	
rat2465	38.15	35.18	35.45	35.93	32.06	31.60	31.89	29.86	33.91	39.07	33.84	36.84	34.11	
rGAPDH	19.25	19.70	19.45	19.21	19.93	19.67	19.30	18.89	19.23	20.93	20.47	20.84	20.68	
Rel EXP	0.02	0.22	0.15	0.09	2.23	2.56	1.62	4.99	0.38	0.03	0.94	0.15	0.91	

Fig. 8

Fig. 9



	rat2465	rGAPDH	Rel EXP
CCL4 24h After Final dose 1	31	19	3
2	28	18	8
3	29	17	3
4	29	18	4
5	30	18	3
6	30	17	2
7	31	18	1
9	31	18	2
10	29	18	3
48h After Final dose 11	29	17	4
12	28	17	7
13	29	18	4
14	32	17	0
15	30	18	2
16	29	17	4
17	29	17	3
18	29	18	5
19	29	18	4
20	30	18	1
96h After Final dose 21	32	19	1
22	31	19	2
23	32	18	1
24	31	17	1
26	32	19	1
27	31	18	1
28	32	20	2
29	32	19	1
30	32	19	2
Control for CCL 4-31	37	19	0
Control for CCL 4-32	36	19	0
Control for CCL 4-33	36	19	0
Control for CCL 4-34	34	19	0
Control for CCL 4-35	37	19	0
Control for CCL 4-36	35	19	0
Control for CCL 4-37	34	18	0
Control for CCL 4-38	37	19	0
Control for CCL 4-39	36	18	0
Control for CCL 4-40	37	19	0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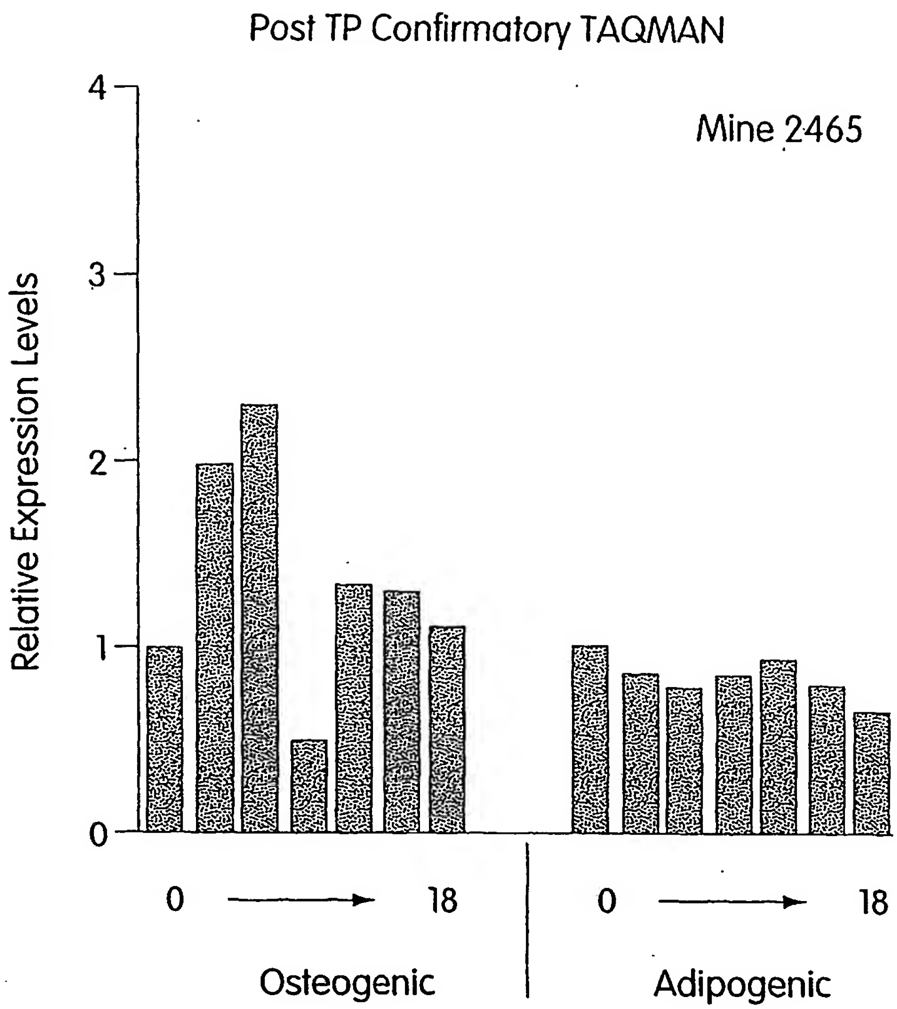
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Adipocyte Lineage
Osteoblast Lineage
Progenitor Lineage

Fig. 10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Mine 2465
Relative Expression Levels
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3
2
1
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18
0
18
Osteogenic
Adipogenic

Fig. 11

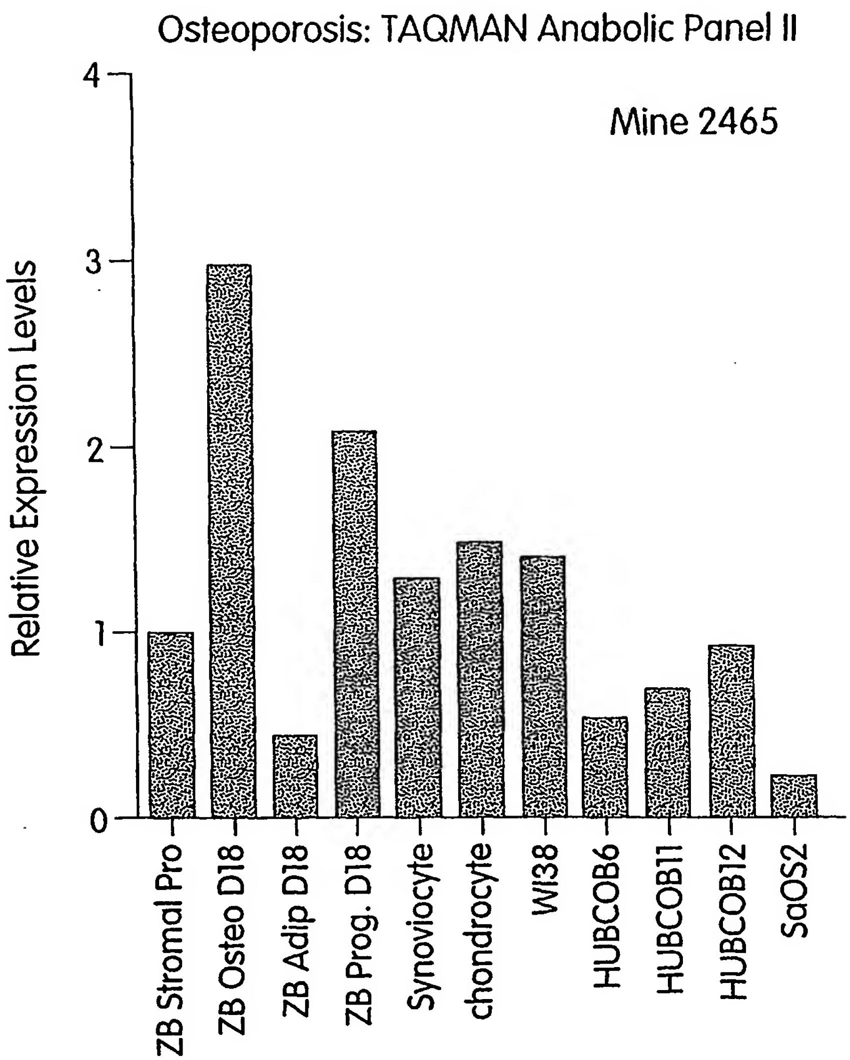
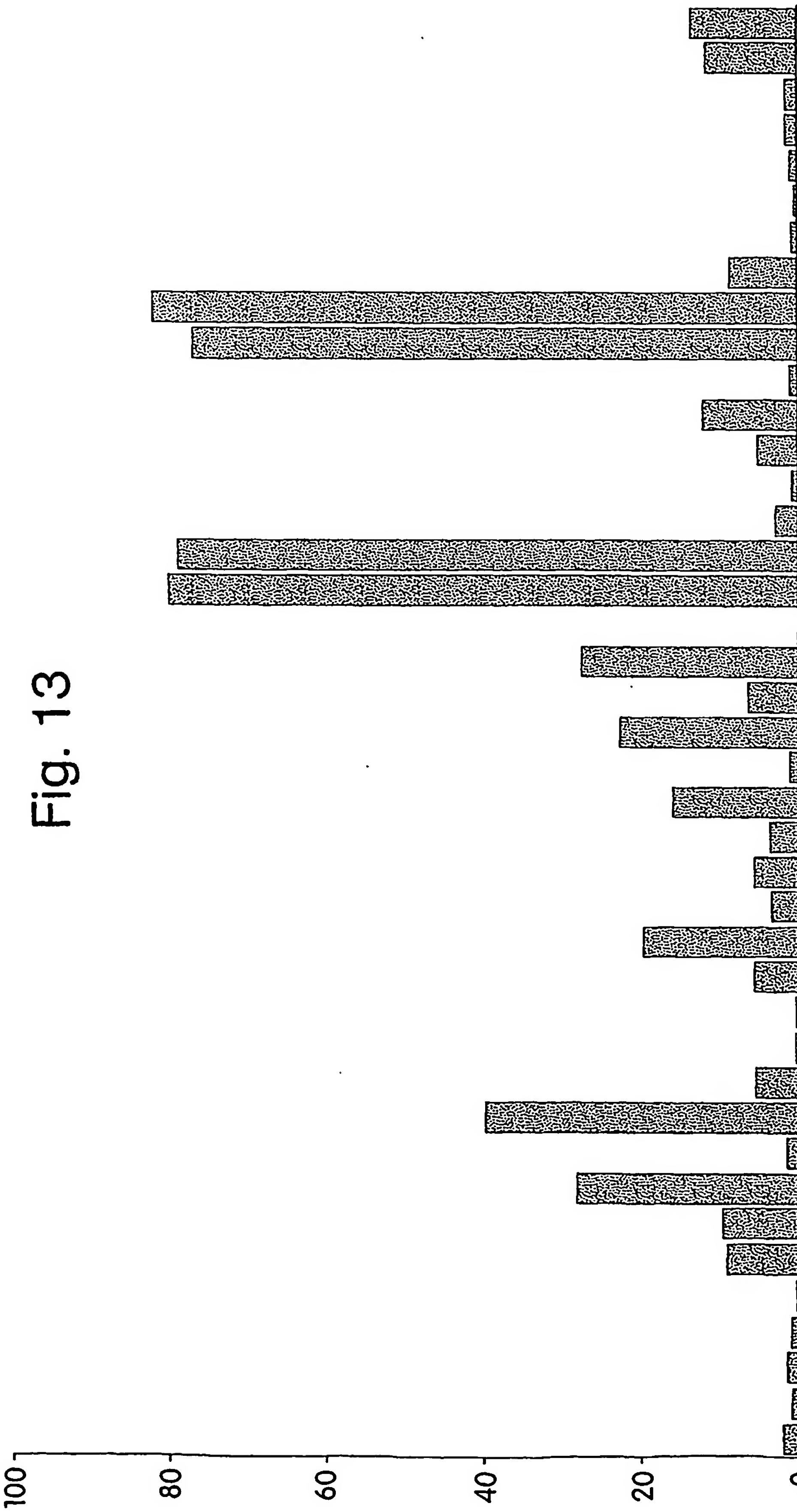

Osteoporosis: TAQMAN Anabolic Panel II
Mine 2465
Relative Expression Levels
4
3
2
1
0
ZB Stromal Pro
ZB Osteo D18
ZB Adip D18
ZB Prog. D18
Synoviocyte
chondrocyte
WI38
HUBCOB6
HUBCOB11
HUBCOB12
SaOS2

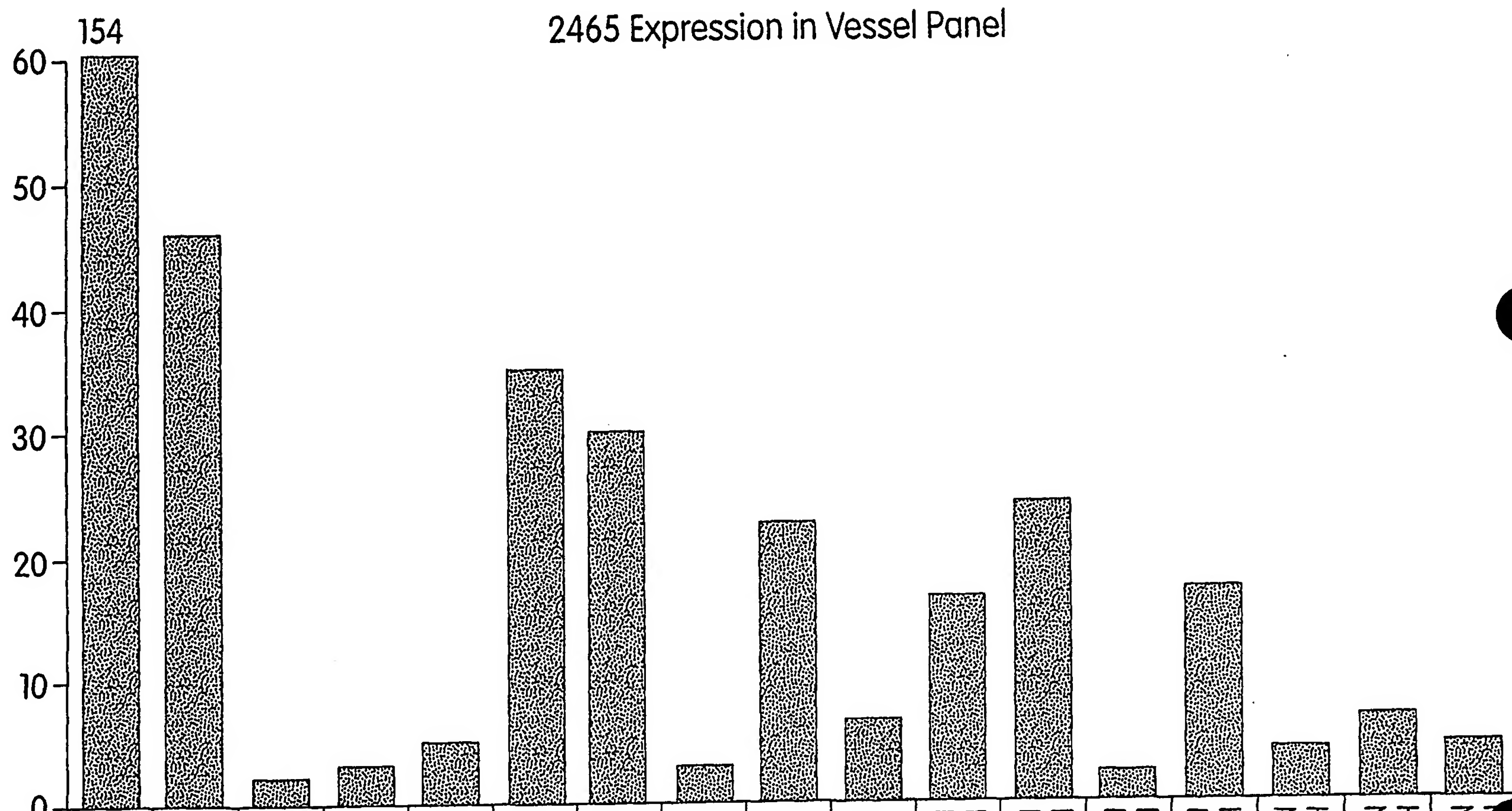
Fig. 12



	5 Average	Average	Delta	REL
Lymph Node MPI	24.1	17.9	6.2	13.2
Tonsil MPI 396	27.4	20.9	6.5	11.4
Thymus MPI 388	28.9	19.4	9.6	1.3
Spinal Cord MPI 651	27.5	18.2	9.3	1.5
Spleen MPI 380	28.2	18.2	9.9	1.0
Cervix MPI 567	31.3	19.5	11.3	0.3
Spinal cord MPI 655	29.7	19.4	10.3	0.8
Prim Cult Osteoblasts	25.3	18.5	6.8	8.7
Dif Osteoblasts	22.7	19.1	3.6	81.9
Fetal Heart MPI 164	31.1	21.0	10.1	0.9
Fetal Heart MPI 32	25.5	19.1	6.4	11.9
Fetal Liver MPI 133	29.7	22.1	7.7	5.0
Fetal Liver MPI 425	29.2	18.8	10.4	0.7
Placenta MPI 391/7	31.8	23.3	8.5	2.7
Testis MPI 33/78	24.1	20.5	3.7	78.8
Skin MPI 572	24.2	20.6	3.6	80.0
Bone Marrow	34.9	20.1	14.8	0.0
Thyroid MPI 54	27.5	22.3	5.2	27.0
Sm Intestine MPI	29.9	22.5	7.4	6.0
Adipose MPI 620	27.2	21.7	5.5	22.3
Adipose MPI 621	29.7	19.4	10.4	0.8
Vein MPI 135	28.9	22.8	6.0	15.4
Vein MPI 134	28.5	20.2	8.3	3.3
Lung MPI 131	27.6	20.0	7.5	5.4
Lung MPI 28	25.3	17.0	8.3	3.2
Kidney MPI 58	26.7	21.0	5.7	19.3
Kidney MPI 27	27.1	19.5	7.6	5.3
Ovary MPI 415	33.7	20.7	13.0	0.1
Ovary MPI 423	32.5	20.5	12.0	0.2
Heart MPI 664	27.1	22.5	4.7	39.8
Colon MPI 176	36.0	26.7	9.3	1.6
Brain MPI 515	26.5	21.4	5.2	28.2
Sk. Muscle MPI	24.4	17.7	6.7	9.8
Sk. Muscle MPI	28.0	21.3	6.8	9.2
Breast	35.4	23.2	12.2	0.2
Liver MPI 75	31.3	21.3	10.0	1.0
Liver MPI 154	32.7	23.3	9.4	1.5
Osteoclasts	25.4	23.8	1.6	1.0
Prostate MPI 412	29.8	20.8	9.0	1.9

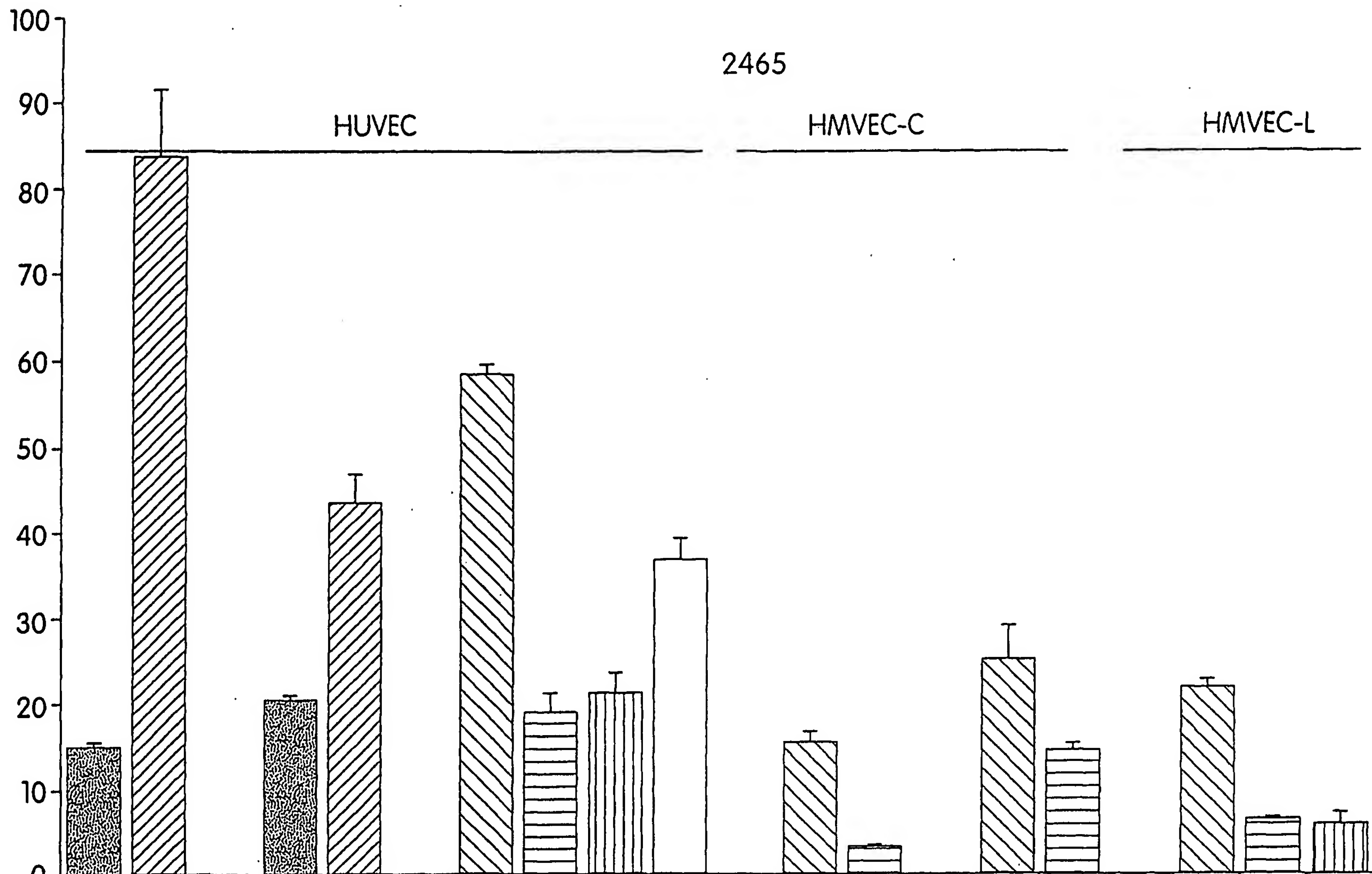
Fig. 13

2465 Expression in Vessel Panel



	Aortic SMC	HMVEC	H/Adipose	H/Artery/ Normal/C	H/Artery/ Normal/C	H/Artery/ Normal/M	H/Artery/ Diseased/III	H/Artery/ Diseased/Tl	H/Aorta/ Diseased	H/Vein/ Normal/Sap	H/Vein/ Normal/Sap	H/Vein/ Normal/Sap	H/Vein/ Normal/Sap	H/Vein/ Diseased/Sa	H/Vein/ Normal/	H/Vein/ Normal/Sap	H/Vein/ Normal/
2465 Mean	24.265	23.35	28.43	27.645	28.475	26.165	24.45	28.81	27.29	28.665	24.5	22.99	28.94	25.28	29.085	27.5	30.015
B2 Mean	22.57	18.905	19.66	19.3	20.825	21.31	19.37	20.37	21.82	21.425	18.565	17.615	20.275	19.415	21.21	20.32	22.235
dCt	2.695	4.445	8.77	8.345	7.65	4.855	5.08	8.44	5.47	7.24	5.935	5.375	8.665	5.865	7.875	7.18	7.78
RE	154.43	45.91	2.29	3.08	4.98	34.55	29.56	2.88	22.56	6.62	16.35	24.10	2.46	17.16	4.26	6.90	4.55

Fig. 14



	stat	LSS		stat	LSS		prolif	conf	-GF	IL-1		prolif	conf		prolif	conf		prolif	conf	-GF
2465 avg Ct	27.22	24.315		27.7	27.18		26.215	26.545	26.385	24.615		26.455	30.15		28.45	28.145		28.445	29.33	29.655
B2M avg Ct	21.155	20.73		22.095	22.655		22.11	20.82	20.815	19.84		20.43	21.8		23.12	22.02		22.905	22.02	22.155
□Expression	14.952	83.696		20.552	43.55		58.122	19.015	21.18	36.604		15.397	3.0836		25.15	14.348		21.511	6.3043	5.6694

Fig. 15

SEQUENCE LISTING

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<120> METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR, HEPATIC, AND BONE DISEASE

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cgcggagccg gagtcccagc ctcgcc atg gga cat aac ggg agc tgg atc tct  893
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cca aat gcc agc gag ccg cac aac gcg tcc ggc gcc gag gct gcg ggt     941
Pro Asn Ala Ser Glu Pro His Asn Ala Ser Gly Ala Glu Ala Ala Gly
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gtg aac cgc agc gcg ctc ggg gag ttc ggc gag gcg cag ctg tac cgc     989
Val Asn Arg Ser Ala Leu Gly Glu Phe Gly Glu Ala Gln Leu Tyr Arg
                 30                  35                  40

cag ttc acc acc acc gtg cag gtc gtc atc ttc ata ggc tcg ctg ctc    1037
Gln Phe Thr Thr Thr Val Gln Val Val Ile Phe Ile Gly Ser Leu Leu
             45                  50                  55

gga aac ttc atg gtg tta tgg tca act tgc cgc aca acc gtg ttc aaa    1085
Gly Asn Phe Met Val Leu Trp Ser Thr Cys Arg Thr Thr Val Phe Lys
         60                  65                  70

tct gtc acc aac agg ttc att aaa aac ctg gcc tgc tcg ggg att tgt    1133
Ser Val Thr Asn Arg Phe Ile Lys Asn Leu Ala Cys Ser Gly Ile Cys
     75                  80                  85

gcc agc ctg gtc tgt gtg ccc ttc gac atc atc ctc agc acc agt cct    1181
Ala Ser Leu Val Cys Val Pro Phe Asp Ile Ile Leu Ser Thr Ser Pro
 90                  95                 100                 105

cac tgt tgc tgg tgg atc tac acc atg ctc ttc tgc aag gtc gtc aaa    1229
His Cys Cys Trp Trp Ile Tyr Thr Met Leu Phe Cys Lys Val Val Lys
                110                 115                 120

ttt ttg cac aaa gta ttc tgc tct gtg acc atc ctc agc ttc cct gct    1277
Phe Leu His Lys Val Phe Cys Ser Val Thr Ile Leu Ser Phe Pro Ala
            125                 130                 135

att gct ttg gac agg tac tac tca gtc ctc tat cca ctg gag agg aaa    1325
Ile Ala Leu Asp Arg Tyr Tyr Ser Val Leu Tyr Pro Leu Glu Arg Lys
        140                 145                 150

ata tct gat gcc aag tcc cgt gaa ctg gtg atg tac atc tgg gcc cat    1373
Ile Ser Asp Ala Lys Ser Arg Glu Leu Val Met Tyr Ile Trp Ala His
    155                 160                 165

gca gtg gtg gcc agt gtc cct gtg ttt gca gta acc aat gtg gct gac    1421
Ala Val Val Ala Ser Val Pro Val Phe Ala Val Thr Asn Val Ala Asp
170                 175                 180                 185

atc tat gcc acg tcc acc tgc acg gaa gtc tgg agc aac tcc ttg ggc    1469
Ile Tyr Ala Thr Ser Thr Cys Thr Glu Val Trp Ser Asn Ser Leu Gly
                190                 195                 200

cac ctg gtg tac gtt ctg gtg tat aac atc acc acg gtc att gtg cct    1517
His Leu Val Tyr Val Leu Val Tyr Asn Ile Thr Thr Val Ile Val Pro
            205                 210                 215

gtg gtg gtg gtg ttc ctc ttc ttg ata ctg atc cga cgg gcc ctg agt    1565
Val Val Val Val Phe Leu Phe Leu Ile Leu Ile Arg Arg Ala Leu Ser
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gcc agc cag aag aag aag gtc atc ata gca gcg ctc cgg acc cca cag    1613
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aac acc atc tct att ccc tat gcc tcc cag cgg gag gcc gag ctg cac    1661
Asn Thr Ile Ser Ile Pro Tyr Ala Ser Gln Arg Glu Ala Glu Leu His
250                 255                 260                 265

gcc acc ctg ctc tcc atg gtg atg gtc ttc atc ttg tgt agc gtg ccc    1709
Ala Thr Leu Leu Ser Met Val Met Val Phe Ile Leu Cys Ser Val Pro
                270                 275                 280

tat gcc acc ctg gtc gtc tac cag act gtg ctc aat gtc cct gac act    1757
Tyr Ala Thr Leu Val Val Tyr Gln Thr Val Leu Asn Val Pro Asp Thr
            285                 290                 295

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Ser Val Phe Leu Leu Leu Thr Ala Val Trp Leu Pro Lys Val Ser Leu
        300                 305                 310

ctg gca aac cct gtt ctc ttt ctt act gtg aac aaa tct gtc cgc aag    1853
Leu Ala Asn Pro Val Leu Phe Leu Thr Val Asn Lys Ser Val Arg Lys
    315                 320                 325

tgc ttg ata ggg acc ctg gtg caa cta cac cac cgg tac agt cgc cgt    1901
Cys Leu Ile Gly Thr Leu Val Gln Leu His His Arg Tyr Ser Arg Arg
330                 335                 340                 345

aat gtg gtc agt aca ggg agt ggc atg gct gag gcc agc ctg gaa ccc    1949
Asn Val Val Ser Thr Gly Ser Gly Met Ala Glu Ala Ser Leu Glu Pro
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agc ata cgc tcg ggt agc cag ctc ctg gag atg ttc cac att ggg cag    1997
Ser Ile Arg Ser Gly Ser Gln Leu Leu Glu Met Phe His Ile Gly Gln
            365                 370                 375

cag cag atc ttt aag ccc aca gag gat gag gaa gag agt gag gcc aag    2045
Gln Gln Ile Phe Lys Pro Thr Glu Asp Glu Glu Glu Ser Glu Ala Lys
        380                 385                 390

tac att ggc tca gct gac ttc cag gcc aag gag ata ttt agc acc tgc    2093
Tyr Ile Gly Ser Ala Asp Phe Gln Ala Lys Glu Ile Phe Ser Thr Cys
    395                 400                 405

ctg gag gga gag cag ggg cca cag ttt gcg ccc tct gcc cca ccc ctg    2141
Leu Glu Gly Glu Gln Gly Pro Gln Phe Ala Pro Ser Ala Pro Pro Leu
410                 415                 420                 425

agc aca gtg gac tct gta tcc cag gtg gca ccg gca gcc cct gtg gaa    2189
Ser Thr Val Asp Ser Val Ser Gln Val Ala Pro Ala Ala Pro Val Glu
                430                 435                 440

cct gaa aca ttc cct gat aag tat tcc ctg cag ttt ggc ttt ggg cct    2237
Pro Glu Thr Phe Pro Asp Lys Tyr Ser Leu Gln Phe Gly Phe Gly Pro
            445                 450                 455

ttt gag ttg cct cct cag tgg ctc tca gag acc cga aac agc aag aag    2285
Phe Glu Leu Pro Pro Gln Trp Leu Ser Glu Thr Arg Asn Ser Lys Lys
        460                 465                 470
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cgg ctg ctt ccc ccc ttg ggc aac acc cca gaa gag ctg atc cag aca    2333
Arg Leu Leu Pro Pro Leu Gly Asn Thr Pro Glu Glu Leu Ile Gln Thr
    475                 480                 485

aag gtg ccc aag gta ggc agg gtg gag cgg aag atg agc aga aac aat    2381
Lys Val Pro Lys Val Gly Arg Val Glu Arg Lys Met Ser Arg Asn Asn
490                 495                 500                 505

aaa gtg agc att ttt cca aag gtg gat tcc tagcaaggat tgtaaattct      2431
Lys Val Ser Ile Phe Pro Lys Val Asp Ser
                510                 515

tggaagcaac ggggggcttc catattccca ccagagtgtg ggaatgctgt ggccatgtga 2491

ttgtatgatc tccttgcaac tcagtgtgag ttgattcctc caatatgggc cagatgcttt 2551

tgaatgatag ggaaatctac ataaaatcca gtgtcctctt tattgaggga gtatatgtat 2611

ccatctcagt gatccatgtc cttagtgaag tccacattat tctctgtggg gacaagagct 2671

gggcagtttt gaatgggtct tgaggtgggt accccatgtg cactttctga ggatgcctca 2731

cttccctggg ctctgcagag aacacacaga gagaagactt tcagagctca caggagcagg 2791

gagcaggagc actctaaggg aattc                                       2816


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<213> Homo sapiens

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Asn Ala Ser Gly Ala Glu Ala Ala Gly Val Asn Arg Ser Ala Leu Gly
             20                  25                  30

Glu Phe Gly Glu Ala Gln Leu Tyr Arg Gln Phe Thr Thr Thr Val Gln
         35                  40                  45

Val Val Ile Phe Ile Gly Ser Leu Leu Gly Asn Phe Met Val Leu Trp
     50                  55                  60

Ser Thr Cys Arg Thr Thr Val Phe Lys Ser Val Thr Asn Arg Phe Ile
 65                  70                  75                  80

Lys Asn Leu Ala Cys Ser Gly Ile Cys Ala Ser Leu Val Cys Val Pro
                 85                  90                  95

Phe Asp Ile Ile Leu Ser Thr Ser Pro His Cys Cys Trp Trp Ile Tyr
            100                 105                 110

Thr Met Leu Phe Cys Lys Val Val Lys Phe Leu His Lys Val Phe Cys
        115                 120                 125

Ser Val Thr Ile Leu Ser Phe Pro Ala Ile Ala Leu Asp Arg Tyr Tyr
    130                 135                 140
```

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Ser Val Leu Tyr Pro Leu Glu Arg Lys Ile Ser Asp Ala Lys [illegible] Arg
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Glu Leu Val Met Tyr Ile Trp Ala His Ala Val Val Ala Ser Val Pro
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Val Phe Ala Val Thr Asn Val Ala Asp Ile Tyr Ala Thr Ser Thr Cys
            180                 185                 190

Thr Glu Val Trp Ser Asn Ser Leu Gly His Leu Val Tyr Val Leu Val
        195                 200                 205

Tyr Asn Ile Thr Thr Val Ile Val Pro Val Val Val Val Phe Leu Phe
    210                 215                 220

Leu Ile Leu Ile Arg Arg Ala Leu Ser Ala Ser Gln Lys Lys Lys Val
225                 230                 235                 240

Ile Ile Ala Ala Leu Arg Thr Pro Gln Asn Thr Ile Ser Ile Pro Tyr
                245                 250                 255

Ala Ser Gln Arg Glu Ala Glu Leu His Ala Thr Leu Leu Ser Met Val
            260                 265                 270

Met Val Phe Ile Leu Cys Ser Val Pro Tyr Ala Thr Leu Val Val Tyr
        275                 280                 285

Gln Thr Val Leu Asn Val Pro Asp Thr Ser Val Phe Leu Leu Leu Thr
    290                 295                 300

Ala Val Trp Leu Pro Lys Val Ser Leu Leu Ala Asn Pro Val Leu Phe
305                 310                 315                 320

Leu Thr Val Asn Lys Ser Val Arg Lys Cys Leu Ile Gly Thr Leu Val
                325                 330                 335

Gln Leu His His Arg Tyr Ser Arg Arg Asn Val Val Ser Thr Gly Ser
            340                 345                 350

Gly Met Ala Glu Ala Ser Leu Glu Pro Ser Ile Arg Ser Gly Ser Gln
        355                 360                 365

Leu Leu Glu Met Phe His Ile Gly Gln Gln Gln Ile Phe Lys Pro Thr
    370                 375                 380

Glu Asp Glu Glu Glu Ser Glu Ala Lys Tyr Ile Gly Ser Ala Asp Phe
385                 390                 395                 400

Gln Ala Lys Glu Ile Phe Ser Thr Cys Leu Glu Gly Glu Gln Gly Pro
                405                 410                 415

Gln Phe Ala Pro Ser Ala Pro Pro Leu Ser Thr Val Asp Ser Val Ser
            420                 425                 430

Gln Val Ala Pro Ala Ala Pro Val Glu Pro Glu Thr Phe Pro Asp Lys
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Tyr Ser Leu Gln Phe Gly Phe Gly Pro Phe Glu Leu Pro Pro Gln Trp
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Leu Ser Glu Thr Arg Asn Ser Lys Lys Arg Leu Leu Pro Pro Leu Gly
465 470 475 480

Asn Thr Pro Glu Glu Leu Ile Gln Thr Lys Val Pro Lys Val Gly Arg
485 490 495

Val Glu Arg Lys Met Ser Arg Asn Asn Lys Val Ser Ile Phe Pro Lys
500 505 510

Val Asp Ser
515

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— *with international search report*

(88) Date of publication of the international search report: 7 March 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/64872 A3

(54) Title: GENE 2465: METHODS AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF CARDIOVASCULAR, HEPATIC AND BONE DISEASE

(57) Abstract: The present invention relates to methods and compositions for the diagnosis and treatment of hepatic, bone, or cardiovascular disorders. Specifically, the present invention identifies 2465 genes which are differentially expressed in hepatic, bone, or cardiovascular disorder states, relative to their expression in normal, or non-hepatic, non-bone, or non-cardiovascular disorder states, and/or in response to manipulations relevant to hepatic, bone, or cardiovascular disorders. The present invention describes methods for the diagnostic evaluation and prognosis of various hepatic, bone, or cardiovascular disorders, and for the identification of subjects exhibiting a predisposition to such conditions. The present invention also provides methods for the identification and

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C12N1/21 C12N5/10 C07K16/18
G01N33/68 [illegible]1/68 A61K38/17 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, SEQUENCE SEARCH, BIOSIS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HATA S ET AL: "CDNA CLONING OF A PUTATIVE G PROTEIN-COUPLED RECEPTOR FROM BRAIN" BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 1261, 1995, pages 121-125, XP000914574 ISSN: 0006-3002 * 100% identity in 515 aa overlap with sequence ID no.2 * * 100% identity in 2816 nt overlap with sequence ID no.1 * the whole document ---	1-22

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2001	09/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/06145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! HSG551, accession number G28551, 12 July 1996 (1996-07-12) R.M. MYERS : "Human STS SHGC-31667, sequence tagged site." XP002178079 * 100% identity in 2816 nt overlap with sequence ID no.1 (1-2816:1-2816) * abstract & UNPUBLISHED, ---	1-7,9-12
X	DATABASE EMBL 'Online! AC012377, accession number AC012377, 27 October 1999 (1999-10-27) L. ROYEN ET AL: "Homo sapiens chromosome 15 clone RP11-325N19 map 15q14, complete sequence" XP002178080 * 99,9% identity in 1528 nt overlap with sequence ID no.1 (2816-1289:39863-41390)* & UNPUBLISHED, ---	1-7
P,X	WO 00 22129 A (ARENA PHARMACEUTICALS INC ;LIAW CHEN W (US); BEHAN DOMINIC P (US);) 20 April 2000 (2000-04-20) * 100% identity in 515 aa overlap with sequence ID no.220 (1-515:1-515) * * 99,9% identity in 1548 nt overlap with sequence ID no.1 (867-2414:1-1548) * Sequences ID no.219 and no.220 page 182-183 page 7; table A page 69; example 6; table F page 70 -page 71 claims -----	1-35

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 25 (as far as an in vivo method is concerned) and claims 30-35 are directed to a method of treatment of the human/animal body (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition as far as the modulator and/or the compound which binds the polypeptide is an antibody.

Continuation of Box I.2

Claims Nos.: 17, 19 ,25, 30-35 all partially

Claims 17and 19 have been searched as far as the compound "which selectively binds to the polypeptide" is an antibody as mentioned in claim 18.

Claim 25 refers to a method of modulating the activity of the polypeptide 2465 comprising contacting said polypeptide with a compound which binds to the polypeptide to modulate the activity of this polypeptide and claims 30-35 refer to a method of treatment comprising administering a modulator of the polypeptide 2465 without giving a a true technical characterization. Moreover, no such specific compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).
No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved. But a partial search has been carried out as far as the "compound which binds " and/or the modulator is an antibody.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/06145

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0022129 A	20-04-2000	AU 6299199 A	01-05-2000
		AU 6430799 A	01-05-2000
		EP 1121431 A1	08-08-2001
		WO 0021987 A2	20-04-2000
		WO 0022129 A1	20-04-2000
		WO 0022131 A2	20-04-2000